

Genomic conflict in scale insects: the causes and consequences of bizarre genetic systems

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ABSTRACT

It is now clear that mechanisms of sex determination are extraordinarily labile, with considerable variation across all taxonomic levels. This variation is often expressed through differences in the genetic system (XX-XY, XX-XO, haplodiploidy, and so on). Why there is so much variation in such a seemingly fundamental process has attracted much attention, with recent ideas concentrating on the possible role of genomic conflicts of interest. Here we consider the role of inter- and intra-genomic conflicts in one large insect taxon: the scale insects. Scale insects exhibit a dizzying array of genetic systems, and their biology promotes conflicts of interest over transmission and sex ratio between male- and female-expressed genes, parental- and offspring-expressed genes (both examples of intra-genomic conflict) and between scale insects and their endosymbionts (inter-genomic conflict). We first review the wide range of genetic systems found in scale insects and the possible evolutionary transitions between them. We then outline the theoretical opportunities for genomic conflicts in this group and how these might influence sex determination and sex ratio. We then consider the evidence for these conflicts in the evolution of sex determination in scale insects. Importantly, the evolution of novel genetic systems in scale insects has itself helped create new conflicts of interest, for instance over sex ratio. As a result, a major obstacle to our understanding of the role of conflict in the evolution of sex-determination and genetic systems will be the difficulty in identifying the direction of causal relationships. We conclude by outlining possible experimental and comparative approaches to test more effectively how important genomic conflicts have been.

Key words: genomic conflict, Coccoidea, sex allocation, genetic system, sex determination, endosymbiont, scale insect.

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I. INTRODUCTION

Genetic systems vary widely, including the presence or absence of sex chromosomes, the number and sex specificity of those chromosomes, the developmental requirement for both parental sets of chromosomes, variation in levels of ploidy between the sexes and sometimes even the complete absence of sexual reproduction (Normark, 2003). The genetic system of an organism provides the context for the evolution of several fundamental biological processes, including sexual *versus* asexual reproduction, sex determination, and many aspects of genome evolution (Bull, 1983; Lynch, 2008; Maynard Smith, 1978). As such, we may expect genetic systems to be the firm foundations on which these other processes evolve. However, it is becoming abundantly clear that genetic systems themselves can be remarkably labile within and among closely related species (Bull, 1983; Normark, 2003). Similarly, determining which sex an organism develops into would appear, at first glance, to be a fundamental developmental process for sexually reproducing organisms. As such, the mechanisms underlying that developmental decision should perhaps be conserved across broad taxonomic groups. Again, however, it is now obvious that there is an extraordinary diversity of sex-determination systems across all levels of taxonomic diversity, including within single species such as the housefly *Musca domestica* (Dubendorfer *et al.*, 2002; Kozielska *et al.*, 2006; Uller *et al.*, 2007). This diversity is expressed at the molecular level as changes in the sex determination cascade (Evans, 2004; Marin & Baker, 1998; Van Doorn & Kirkpatrick, 2007), but is also reflected in genomic terms as variation in the genetic systems of closely related organisms (Normark, 2003).

Understanding the observed diversity of genetic systems across taxa has become an important avenue of research in evolutionary biology, with a central question being why do some groups of species vary tremendously in their genetic systems, whilst in other groups the genetic system is rather more conserved (e.g. female heterogamety across birds, or across Lepidoptera)? Currently, the idea that various forms of genomic conflict shape genetic systems is becoming increasingly influential (Burt & Trivers, 2006; Haig, 1993;

Normark, 2004a). Genomic conflict refers to conflicts of interest between different genetic entities over the state of a given trait, typically related to reproduction. Under conflict, selection favours different trait values for the different entities (Leigh, 1971). These different genetic entities may share a genome (intra-genomic conflict), with conflicts of interest between males and females (sexual conflict: Arnqvist & Rowe, 2005; Parker, 1979) and between parents and offspring (Godfray, 1995; Trivers, 1974) being the most familiar examples. Alternatively, the different genetic entities may be genomically isolated from each other, as with conflicts between hosts and endosymbiotic organisms (inter-genomic conflict). Whilst this separation is useful, conflicts with some reproductive parasites such as transposable elements could be viewed plausibly as either (Burt & Trivers, 2006).

In this review, we will consider whether genomic conflict can explain the observed patterns of the evolution of genetic systems. We will focus on two broad classes of conflict, those concerned with the genetic drive of selfish genetic elements of one sort or another, which can result in the production of biased sex ratios, and those concerned with more direct conflicts over sex determination and sex ratio. As such, sex determination and biased sex ratios will form a common thread running through the article. Both types of genomic conflict can occur within genomes, as well as among genomes, and we will address both. The context for this review will be the extraordinary diversity of genetic systems and modes of reproduction in one group of insects: the scale insects.

Scale insects (Hemiptera: Sternorrhyncha: Coccoidea) have one of the largest varieties of genetic and sex determination systems (Gullan & Kosztarab, 1997; Hughes-Schrader, 1948; Nur, 1980). Often, closely related species with very similar life histories differ in their genetic system. This makes them an ideal group to explore the evolution of different genetic/sex determining systems. Genomic conflict has been suggested to play an important role in the evolution of scale insects and their diverse genetics (Burt & Trivers, 2006; Normark, 2004a; Ross & Shuker, 2009). We will ask how well genetic drive and conflicts over sex determination and sex allocation can explain scale insect genetic systems, but we will also consider how genomic conflicts over transmission and reproduction feed back on each other,

creating new opportunities for conflict to arise. These interactions make inference problematic, and whenever possible we highlight to what extent empirical work can unravel these complexities.

We begin by introducing the problem: the diversity of scale insect genetic systems. We then consider more traditional explanations for these patterns, before introducing and discussing intra- and inter-genomic conflicts over transmission and sex ratio. We finish by asking how best progress can be made in terms of both scale insects and patterns of genetic system evolution more generally.

II. THE BIOLOGY OF SCALE INSECTS

(1) Life history

Scale insects are small plant-feeding insects. They are a superfamily (Coccoidea) of the order Hemiptera, most closely related to the aphids and white-flies. There are more than 7000 species of scale insects described in approximately 28 families. Scale insects have in general a fairly similar (or uniform) life history. In most species, the sexes are indistinguishable as first-instar larvae, with both sexes having well-developed legs and antennae. It is generally assumed that the first-instar larvae (“crawlers”) are the main dispersal stage. The development of males and females is similar until the end of the second instar, when the males pupate and stop feeding. As adults, males are typically fully winged, whilst females are typically wingless, with a mostly (and sometimes completely) sedentary lifestyle. Females have therefore evolved a wide variety of strategies to protect themselves against predators and other environmental influences. Most species form a waxy protective cover over their body (Gullan & Kosztarab, 1997) and species of several families have recruited the plants they infest to help protect them, forming galls on the host plant (Gullan, Miller & Cook, 2005). Adult males locate females using pheromones, although due to their small size, short lifespan and limited flight ability it is not thought that males are able to migrate far (Gullan & Kosztarab, 1997).

Scale insects feed almost exclusively on the phloem of their host plant, forming dense colonies. This is problematic as phloem contains mainly carbohydrates and lacks certain essential amino acids. Scale insects have solved this problem by forming important symbioses with bacteria (and sometimes yeast-like fungi) (Buchner, 1965; Tremblay, 1989), which provide them with the essential nutrients lacking in their diet. We consider the significance of endosymbionts in more detail in Section VI.1. Most scale insects also produce vast amounts of honeydew in order to dispose of the excess sugar in their diet. The colonial habit means that many scale insects species are serious pests on crops and ornamental species the world over, causing severe economic damage (Ben-Dov, Miller & Gibson, 2009). They damage plants directly by feeding, but they can also indirectly cause damage by transmitting plant pathogens through injection

or through the build-up of honeydew that can promote the attack of fungi and other plant pathogens.

(2) The diversity of genetic systems in scale insects

Scale insects display a quite remarkable variety of genetic systems (Fig. 1). Several scale insects have a XX-XO system and this system has been assumed to be ancestral (Nur, 1980). However in many other taxa a variety of different genetic systems has evolved, often more than once. Nur (1980) reviewed the different genetic systems found in scale insects and reconstructed their possible evolutionary history. Fig. 1A presents the possible evolutionary pathways of the different genetic systems based on recent molecular phylogenies of scale insects and also includes recent data on the genetic systems of several scale insect taxa as reviewed by Gavrillov (2008). We review the major transitions in genetic system below, noting immediately the complex patterns of evolutionary loss and gain of different genetic systems. We number each transition as in Fig. 1A.

(3) Evolutionary transitions 1–3: paternal genome elimination

The most widespread and bizarre deviation from the XX-XO genetic system is that represented by paternal genome elimination (PGE) (Nur, 1980). PGE is found in 14 scale insect families, including the economically important mealybugs (Pseudococcidae) and armoured scale insects (Diaspididae) (see Fig. 1B). In this system both sexes develop from fertilized eggs, but during early development of the male offspring the paternal half of the genome is deactivated through heterochromatinization. Although the deactivated set divides faithfully in all somatic cell lines, it fails to end up in mature sperm because it is destroyed during meiosis and is not passed on to the offspring of the male (Schraeder, 1921). Later studies showed that the deactivated genome set was of paternal origin (Brown & Nelson-Rees, 1961).

In the scale insects three different types of PGE are found, that differ in the timing of the loss of the paternal genome set (see Fig. 2). The ancestral system of PGE is the lecanoid system (Transition 1), found in the mealybugs (Pseudococcidae), the lac scale insects (Kerriidae) and some felt scales (Eriococcidae). In this system, although the paternal genome set is deactivated in early development it is only lost during spermatogenesis (see Fig. 2). In the more derived Comstockiella system (Transition 2, named after the genus of armoured scales it was first found in; Brown, 1957) the paternal genome is deactivated at the same time as in the lecanoid system and it is present in all somatic cells. The main difference between the lecanoid and the Comstockiella systems is that in the latter some of the heterochromatinized paternal chromosomes are lost just prior to spermatogenesis (Brown, 1963; Nur, 1980) (see Fig. 2). The remaining paternal chromosomes then undergo the same fate as in the lecanoid system, being separated from the euchromatic chromosomes and failing to end up in the sperm. The number of chromosomes that are lost before spermatogenesis can vary between species,

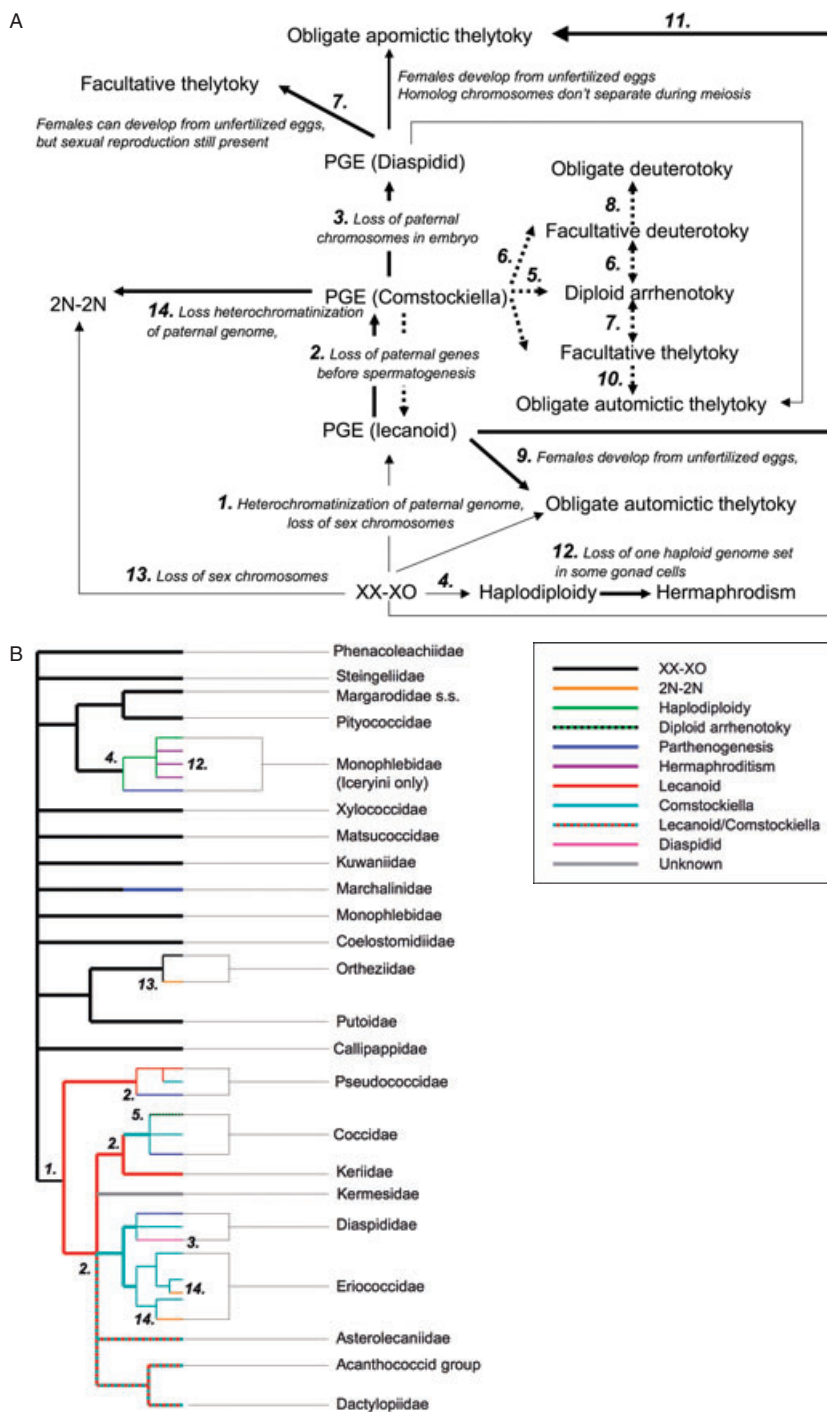


Fig. 1. (A) A schematic overview of the transitions between the different genetic systems across the Coccoidea. The schematic is adjusted from Nur (1980) based on the most recent molecular phylogeny and also including all the asexual systems. Thin arrows represent single transitions while thick arrows represent multiple transitions. Broken arrows represent uncertain or hypothetical transitions. The numbers correspond with the descriptions of the transitions in the text. Those transitions in italics are the most important transitions in the context of this article. PGE refers to paternal genome elimination, while 2N-2N refers to diploidy systems that lack sex chromosomes. All genetic systems are described in more detail in Section II. (B) A family-level phylogenetic reconstruction of the scale insects based on Gullan & Cook (2007). The different coloured lines represent the different genetic systems found across the clade. If there are differences within families this is shown by including a schematic representation of the within-family relationships (the thinner lines, based on (Cook *et al.*, 2002; Downie & Gullan, 2004; Hardy, Gullan & Hodgson, 2008; Unruh & Gullan, 2008). Branch lengths are not to scale. The numbers again represent the transitions described in the text. *Margarodidae s.s.* refers to the *Margarodidae sensu stricto*.

between individuals of the same species and even between different germ line cells within a single individual (as in *Eriococcus araucariae* and *Eriococcus spuriosus* (Fig. 3F); Brown, 1967). In most species about 75% of the heterochromatic set is destroyed before spermatogenesis (Brown, 1967). The Comstockiella system seems to have evolved multiple times from the lecanoid system (see Fig. 1) and there is a suggestion that evolution has gone both ways and some taxa even seem to have a combination of the two systems within individuals (*Eriococcus araucariae* Brown, 1967). The third system of PGE is the Diaspidid system (Transition 3) found in the armoured scales (Diaspididae). In this system the paternal genome does not become heterochromatinized, instead it is lost during early development at about the same time that the paternal chromosomes become heterochromatinized in the two other systems (Nur, 1980) (see Fig. 2). Elimination is accomplished by so-called anaphase lagging of the paternal set, whereby the chromosomes during the anaphase of mitosis do not move to the spindle quickly enough to be incorporated in a new nucleus. Males in the Diaspidid system therefore become completely haploid both in the somatic and germline cells, even though they develop from fertilized eggs. Recent molecular phylogenies of scale insects have confirmed Nur's (1980) original hypothesis that PGE only evolved once in scale insects (Cook, Gullan & Trueman, 2002) and that the more derived Comstockiella and Diaspidid systems have evolved from the lecanoid system (see Fig. 1).

(4) Evolutionary transitions 4 and 5: haplodiploidy and diploid arrhenotoky

True haplodiploidy is found in several species of the genus *Icerya* and seems to have evolved from a XX-XO system (transition 4). In this system females develop from fertilized eggs and are diploid, while males develop from unfertilized eggs and are haploid. In addition to true haplodiploidy, a very similar system has also been found in scale insects: diploid arrhenotoky (transition 5). This system differs from haplodiploidy in that, in the unfertilized eggs that develop into males, diploidy is restored by a fusion of the first haploid cleavage nuclei, so that both sexes are diploid. However, as if this was not complicated enough, shortly after diploidy has been restored in males, one of the chromosome sets becomes heterochromatinized, leaving males again with haploid gene expression. This curious system has so far only been found in one species of soft scale genus *Parthenolecanium* (previously *Lecanium*) (Nur, 1971, 1972). It probably evolved from the PGE system found in related species, although it might also have evolved from one of the parthenogenetic systems that are found in some species of the same genus (see below). What is fascinating about this species is that heterochromatinization happens to one set of chromosomes even though both sets of chromosomes in males presumably are identical (i.e. of maternal origin), both in terms of DNA sequence and any epigenetic marks such as genomic imprints (see Section IV for further discussion of how chromosomes are chosen for heterochromatinization).

(5) Evolutionary transitions 6–11: parthenogenesis

There are six different parthenogenetic systems found in scale insects (extensively reviewed by Nur, 1971). These systems can be broadly divided with respect to three characteristics: (1) whether males are absent or occasionally present (obligate parthenogenesis or facultative parthenogenesis); (2) which sexes can develop from fertilized and unfertilized eggs; (3) how diploidy is restored in unfertilized eggs (see also Table 1).

In species with facultative deuterotoky (transition 6, also see Table 1), reproduction can be both sexual and asexual and both males and females can develop from either fertilized or unfertilized eggs (within the same species). Individuals that develop from unfertilized eggs restore diploidy by fusion of the first haploid cleavage nuclei, resulting in complete homozygosity. In species with facultative thelytoky (transition 7), unfertilized eggs develop into females, fertilized eggs into both sexes. Meiosis is normal and diploidy is restored by fusion of the polar body with the haploid pronucleus. In obligate deuterotoky (transition 8), unfertilized eggs develop into both sexes but males are inviable or sterile and no sexual reproduction seems to take place. Three types of strictly obligate parthenogenetic systems (transitions 9, 10 and 11) are found in scale insects. In all systems females develop from unfertilized eggs and males are absent, the main difference being that in one system (obligate apomictic thelytoky, transition 11), the homologous chromosomes do not separate during meiosis and oogenesis produces diploid eggs. In the other two systems (obligate automictic thelytoky), meiosis is normal and eggs are haploid. Diploidy is restored either by the fusion of the pronucleus and one of the polar bodies (transition 9) or by fusion of the first haploid cleavage nuclei (transition 10). Table 1 summarises the characteristics of the different parthenogenetic systems and gives an overview of the taxa in which these systems are found.

Although parthenogenetic reproduction is common in scale insects and has been found to have evolved independently in many families, only two systems, obligate apomictic thelytoky and obligate automictic thelytoky, have been found outside the soft scales (Coccidae); all other systems described above are only found in a few related genera of soft scale (mainly in the genus *Parthenolecanium*, see Fig. 3A), with different systems often being found in closely related species (Nur, 1971, 1980). Currently very little is known about the evolution of parthenogenesis in scale insects. In order to understand better how the different parthenogenetic systems in scale insects have evolved and why parthenogenesis is both more common and more variable in soft scales than in any other scale insect family, more data are needed from the relevant genera, especially a detailed phylogeny of soft scales.

(6) Evolutionary transition 12: hermaphroditism

Although hermaphroditism is common in many plants, vertebrates and crustaceans, it is extremely rare in insects (Jarne & Auld, 2006; Normark, 2003). The only insect taxa

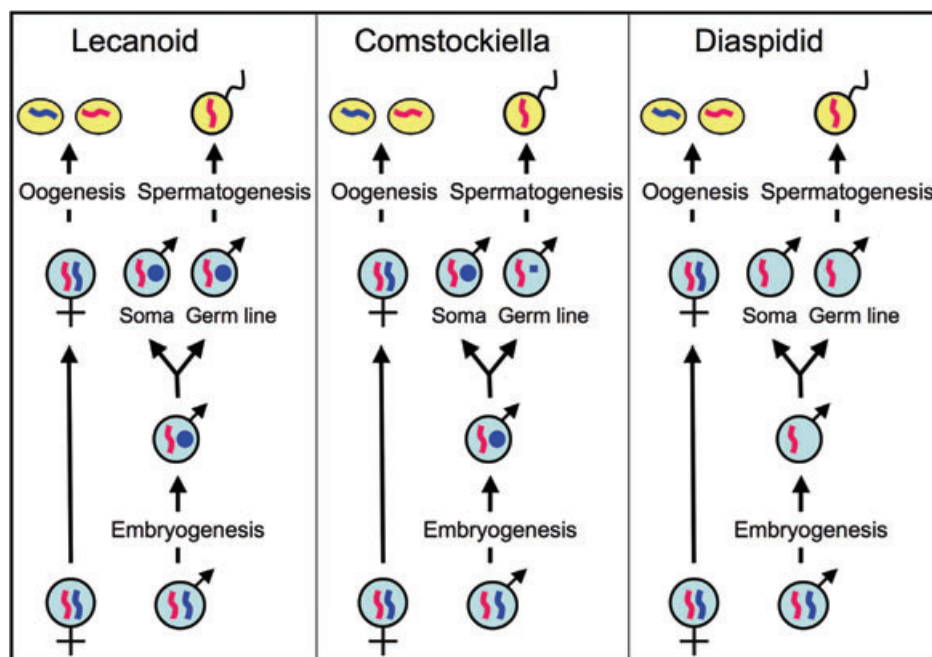


Fig. 2. A schematic representation of the three different paternal genome elimination (PGE) systems found in scale insects: the lecanoid, Comstockiella and the Diaspidid systems. In each figure the pink line represents the maternally inherited chromosome set while the blue line represents the paternally inherited chromosomes. The blue circle represents the heterochromatinized state of the paternally derived genome, while the size of the circle indicates the number of paternal chromosomes.

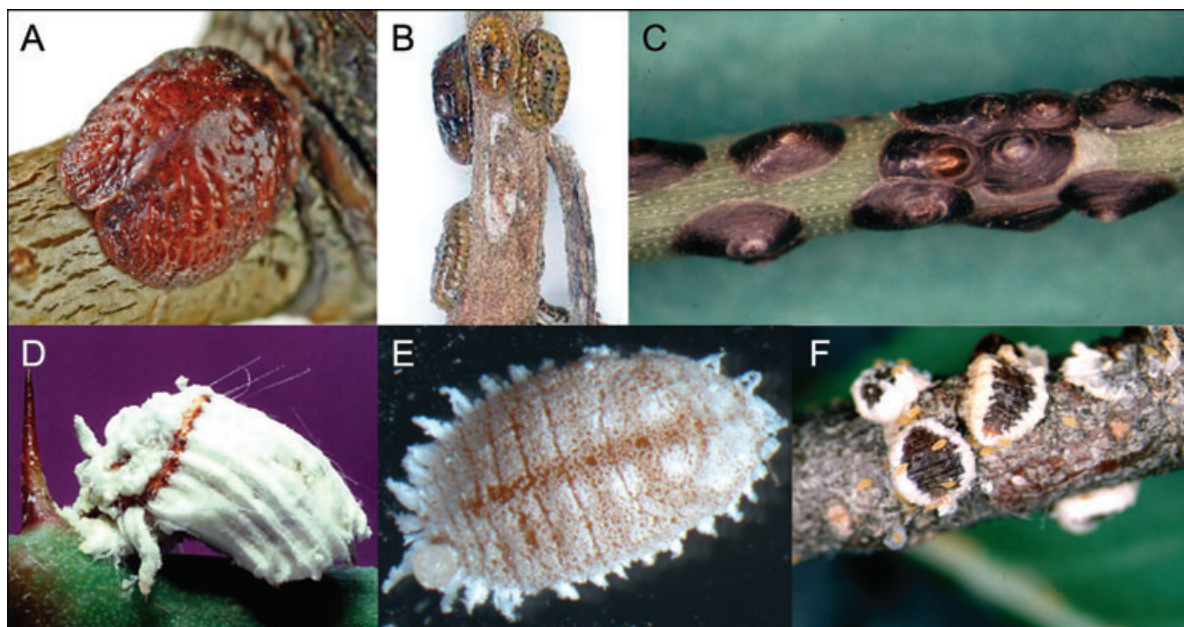


Fig. 3. Scale insects from six different families. (A) Adult female of *Parthenolecanium corni*, a soft scale insect (Hemiptera: Coccidae) which reproduces by obligate automictic thelytoky © Entomart. (B) Adult females of *Stictococcus* sp. (Hemiptera: Stictococcidae). Photo by Alessandra Rung. (C) Adult females of the black pine scale *Dynaspidiotus californicus* on pine needle (Hemiptera: Diaspididae). Picture by Whitney Cranshaw, Colorado State University, Bugwood.org. (D) Adult female with ovisac of the cottony cushion scale *Icerya purchasi* (Hemiptera: Monophlebidae). (E) Adult female of the citrus mealybug *Planococcus citri*. Picture by Laura Ross (F) Adult females and first-instar larvae ("crawlers") of the European elm scale *Eriococcus spuria* (Hemiptera: Eriococcidae). Picture by Whitney Cranshaw, Colorado State University, Bugwood.org.

Table 1. Overview of the different asexual reproductive modes found in the scale insects. For each system the characteristics of the particular form of asexuality and the taxonomic range are shown. Based on Nur (1971).

Genetic system	Reproduction asexual/sexual	Females develop from	Males develop from	Diploidy restored by	Taxonomic range
Facultative deuterotoky	Both	Fertilized and unfertilized eggs	Fertilized and unfertilized eggs	Fusion of the first haploid cleavage nuclei	Coccidae: <i>Parthenolecanium corni</i>
Obligate deuterotoky	Asexual	Fertilized and unfertilized eggs	Fertilized and unfertilized eggs	Fusion of the first haploid cleavage nuclei	Coccidae: <i>Pulvinaria hydrangae</i>
Facultative thelytoky	Both	Fertilized and unfertilized eggs	Fertilized eggs	Fusion polar body with pronucleus	Coccidae: <i>Coccus hesperidum</i> , <i>Saissetia coffeae</i>
Obligate automictic thelytoky (type 1)	Asexual	Unfertilized eggs	Absent	Fusion polar body with pronucleus	Coccidae: <i>Parthenolecanium corni</i> , some populations of: <i>Coccus hesperidum</i> , <i>Saissetia coffeae</i> Pseudococcidae: <i>Phenacoccus solani</i>
Obligate automictic thelytoky (cleavage)	Asexual	Unfertilized eggs	Absent	Fusion of the first haploid cleavage nuclei	Coccidae: 2 species Monophlebidae: <i>Gueriniella serratalae</i>
Obligate apomictic thelytoky	Asexual	Unfertilized eggs	Absent	No meiosis	Coccidae: 2 species Diaspididae: eight species Marchalinidae: <i>Marchalina hellenica</i> Pseudococcidae: two species

where hermaphroditism has been confirmed with certainty are three species of iceryine scale insects: *Icerya purchasi* (Fig. 3D), the African species *Gigantococcus bimaculata* (previously *Icerya*), and the Panamanian species *Crypticerya zeteki* (also previously *Icerya*) (Hughes-Schrader & Monahan, 1966). A recent molecular phylogeny of iceryine scales confirms that these three species constitute three independent origins of hermaphroditism (Unruh & Gullan, 2008). In all these species the hermaphroditic individuals develop from fertilized eggs and are diploid. However, certain cells in the gonad are haploid and these cells proliferate and eventually produce spermatozoa while the diploid cells in the gonad form the oocytes. Most oocytes are fertilized within the body of the hermaphrodite, whilst a small percentage (approximately 10% in *I. purchasi*) of the eggs do not get fertilized and develop into males. These haploid males are viable and are capable of copulating with the hermaphrodites, although it has not been established whether they are able to fertilize eggs (Hughes-Schrader & Monahan, 1966). When this process was first discovered it was assumed that the haploid cells in the gonad originate from diploid cells through the loss of one of the genome sets. However Royer (1975) suggested a different origin. He showed that contrary to what would be expected if the cell originated from genome loss, the haploid cells are present in a newly formed embryo from the moment of fertilization. He also showed that oocytes are often penetrated by multiple sperm (polyspermy) and that although only one of these sperm cells fertilizes the oocyte, several form haploid pronuclei that persist in the embryo. Royer (1975) argued that these haploid sperm pronuclei form the haploid “male” germline. Normark (2009) discusses this remarkable finding as a male adaptation to ensure the fertilization of all oocytes a female carries as well as providing a means of fertilizing all her future female descendants by infecting females with what he calls “transmissible spermatogenic stem cells”. Another interesting observation from this extraordinary system is that the haploid sperm pronuclei tend to develop in close proximity to the host’s endosymbiotic bacteria (Royer, 1975) (see Section VI.5). All other species in the genus *Icerya* that have been studied to date are strictly haplodiploid and no signs of hermaphroditism have been found (Nur, 1980).

(7) Evolutionary transitions 13 and 14: diplodiploidy (XX-XO/ 2N-2N)

There are two different types of diplodiploidy in scale insects. One of them, the XX-XO system, is found in basal scale insects and many other Sternorrhyncha and is therefore assumed to be the ancestral genetic system in scale insects. The second system is a diplodiploid system that lacks the X chromosomes and has evolved an alternative sex determination system (2N-2N: transitions 13 and 14). It has evolved at least three times in scale insects, both directly from the ancestral XX-XO system in the genus *Orthezia* (transitions 13), and from two PGE lineages in the genera *Lachnoidius* and *Stictococcus* (Fig. 3B) respectively (transitions 14) (Normark, 2003; Nur, 1980). Interestingly the loss of

heterochromatinization in males coincides with the loss of endosymbionts in *Stictococcus* males (see Section V.2 and VI.5) (Buchner, 1965).

(8) Explaining the diversity of genetic systems

How can we explain the remarkable diversity of genetic systems in scale insects highlighted above? A number of hypotheses have been formulated to account for the evolution of this diversity and in the next three sections we will consider the most important of them. First, several authors have discussed the possible influence of idiosyncrasies of scale insect biology, highlighting their unusual chromosomes and their life histories. Second, we will consider the more recent ideas concerning genomic conflict, first dealing with intra-genomic conflicts, before moving onto inter-genomic conflicts, and in particular conflicts with endosymbiotic bacteria. It will be important to remember that we seek to explain not only the diversity of genetic systems themselves, but also why we see such a richly dynamic set of transitions between them (Fig. 1). Different genetic systems have evolved multiple times, from different ancestral states, and apparently at different rates. As will become apparent, some hypotheses were formulated for rather specific transitions, while others are more general. Hypotheses specific to certain transitions may be correct, but may be harder to accommodate in a general theory of genetic system evolution that has to explain a diversity of outcomes from a diversity of starting points. Finally, it will also be important to remember that the dynamic pattern of transitions between different systems observed in scale insects makes inference of causation problematic, with selection for the maintenance of a given genetic system likely to differ from the selection underlying the origin of that system (if such selection existed at all).

III. THE POSSIBLE ROLES OF SCALE INSECT BIOLOGY IN THE EVOLUTION OF THEIR GENETIC SYSTEMS

Scale insects have a number of notable biological features that several authors have suggested may explain their rich array of genetic systems. For instance, as in other Hemiptera, scale insects have holokinetic chromosomes that lack a localized site for the attachment of spindle fibres (the centromere) (Hughes-Schrader, 1948). This means that even small, fragmented chromosomes can still regularly divide during cell division. Amongst other things, this might explain the instability in chromosome numbers observed in some scale insect families (e.g. in the genus *Apiomorpha*) (Cook, 2000). In addition to their holokinetic chromosomes however, scale insects also have an inverse meiosis (Chandra, 1962; Hughes-Schrader, 1930; John, 1990). In inverse meiosis, the two sister chromatids disjoin first in meiosis I and only afterwards do the maternal-paternal homolog chromosomes become separated during meiosis II. The four haploid products that are formed

by meiosis I and II do not separate but come to lie in a quadrinucleate spermatid. In taxa with the XX-XO system, all the haploid products form sperm, whilst in the species with PGE only the maternal chromosomes develop into sperm and the paternal chromosome products degenerate. Haig (1993) therefore suggested that this system might predispose scale insects to exhibit genomic drive because the four haploid products of meiosis are contained in the spermatid, giving the opportunity for one set of the chromatids (either the X-bearing or the euchromatinized set) to produce a substance to harm the other set and prevent it from contributing to sperm production. The idea that the combination of holokinetic chromosomes and inverted meiosis might predispose the evolution of new genetic systems is also supported by the presence of PGE-like systems in mites, which are one of the few taxa that share these characteristics with scale insects (Wrensch, Kethley & Norton, 1994). Additionally, a factor that might not explain the evolution of PGE but that might have enabled its evolution is the high level of asynapsis (the failure of homologous chromosomes to pair during meiosis) found in many scale insects (Hughes-Schrader, 1955). This will reduce recombination between maternal and paternal chromosomes, which seems essential for the evolution of PGE (Haig, 1993).

An alternative, but not mutually exclusive, suggestion for the diversity of genetic systems comes from scale insect life history. In all sexually reproducing scale insects there is a strong sexual dimorphism, with adult females being wingless, sessile and covered with protective secretions, while adult males are winged, do not feed as adults and are usually smaller and shorter-lived. Several authors have argued that this difference can lead to a shortage of males (because of their fragility and short lifespan) and that it would therefore be beneficial to evolve reproductive systems that do not depend on males (Hughes-Schrader, 1948). This theory could potentially explain the multiple evolution of parthenogenesis in several scale insects, and possibly also the evolution of true haplodiploidy in some iceryine scale insects. It is however hard to understand how PGE could have evolved and been stable for millions of years (Herrick & Seger, 1999) if scarcity of males was a strong selective pressure. First, the evolution of a reproductive system that depends less on males (like haplodiploidy or parthenogenesis) from PGE seems easy, but it is actually relatively rare (i.e. there are more PGE scale insects than haplodiploid ones and the particular transition has not been observed). Second, one might imagine that it is actually easier to evolve more robust males than to do without males all together. At the very least females could evolve facultative sex allocation such that extra males can be produced if environmental cues suggest that males are likely to be rare (see below for examples of facultative sex allocation). Moreover, it has been noted that the short lifespan and fragility of males in scale insects might themselves have evolved as a response to conflicts between host and endosymbionts, making inferences about the direction of causality problematic (see host-endosymbionts conflicts below).

Finally, Bull (1983) suggested that the evolution of haplodiploidy might be explained by the life history of many of these species. He pointed out that their life history causes high levels of competition between offspring leading to the optimal sex ratios being biased (e.g. Charnov *et al.*, 1981; Hamilton, 1967). He therefore argued that this would lead to strong selection for females to be able to adjust their sex ratio accordingly and that haplodiploidy would allow females to do this, given the apparently straightforward sex determination mechanism of either fertilising eggs or not (making female and male offspring, respectively). He also argued that PGE has strong similarities with haplodiploidy (arrhenotoky) and that both systems are often found in closely related species. Therefore he considered PGE as an intermediate stage in the evolution of haplodiploidy from diploid systems and that PGE systems will evolve towards haplodiploidy (Bull, 1983). Although patterns consistent with this theory have been observed in mites (Cruickshank & Thomas, 1999), there is no evidence for this in scale insects, where haplodiploidy and PGE have both independently evolved from the ancestral XX-XO system (Nur, 1980). The recent finding of female sex ratio control in several species with a PGE system (Nagelkerke & Sabelis, 1998; Ross *et al.*, 2010; Varndell & Godfray, 1996) does suggest however that Bull's (1983) hypothesis that selection for female control over sex allocation on the evolution of both PGE and haplodiploidy is plausible, even if the two systems evolved independently.

Aspects of scale insect biology may thus have influenced the evolution of their genetic systems, at least in terms of making certain transitions more attainable. However, such explanations are by their nature somewhat *post hoc*, and difficult to test in terms of predictions independent of the phenomena they set out to explain. Aspects of scale insect biology such as their inverse meiosis may therefore help us with some of the more proximate or mechanistic explanations for the evolution of the genetic systems, but we might have to look elsewhere for more ultimate, functional explanations.

IV. INTRA-GENOMIC CONFLICTS OVER TRANSMISSION

Several hypotheses for the evolution of the variability of genetic systems in scale insects have been put forward that have gone beyond trying to pin their genetics down to particular aspects of their biology. As such, these theories may also be more generally applicable to species outside the Coccoidea. These hypotheses are based on the idea of evolutionary conflicts of interest between different genetic entities, such as males and females or hosts and endosymbionts (see Section VI). These conflicts of interest are fundamentally associated with how different genetic entities transmit copies of their genes into the next generation. Put another way, genes experience different patterns of selection depending on the context in which they find themselves (males or females, parents or offspring). As we will see, in many cases conflict over genetic transmission will lead to

biases in the sex ratio (including the complete disappearance of males). In other cases, the sex ratio itself is a direct target of conflict. Some of these hypotheses concern the evolution of just one genetic system, while others try to explain the broad diversity of systems itself. In this section, we consider conflict arising within the scale insect genome over transmission, such that changes in the genetic system favour one genetic entity or another. For intra-genomic conflicts, these entities are either males and females, parents and offspring, or sex chromosomes and autosomes.

The first suggestion for an influence of genetic conflict on the evolution of haplodiploidy came from Brown (1963, 1964), who made some of the most important advances in understanding both the mechanisms and diversity of genetic systems in scale insects. He recognized that maternally inherited genes that cause the exclusive transmission of maternal chromosomes during spermatogenesis can have a selective advantage and therefore increase in frequency in the population (Brown, 1964). This idea was further advanced by Bull (1979), who rephrased Brown's (1964) ideas in terms of conflict between maternally and paternally inherited genes. Brown (1964) also proposed a model for the evolution of the different types of PGE, however at that time the Comstockiella system was misinterpreted and therefore we do not include his model in this review. Haig (1993) more recently developed a model showing that X-chromosomal drive in combination with the evolution of maternal autosome X-chromosome linkage could lead to the evolution of PGE (Fig. 4A). This model states that if the evolution of X-chromosomal drive occurred in ancestral scale insects with an XX-XO system, a possible adaptive response of the maternal autosomal genes could have been to evolve a mechanism that would ensure the linkage between the driving X and the maternal autosomes. However this scenario would lead to a strongly female-biased sex ratio and therefore strong selection for an alternative (non-X linked) sex-determining mechanism. The evolution of these two factors, combined with the deactivation of the paternal genome in males, could have led to the evolution of a system similar to lecanoid PGE (see also Fig. 4A).

The previous models all focus on how PGE could have evolved from the ancestral XX-XO chromosome system (transition 1). However, the evolution of PGE across scale insects has involved repeated evolutionary events producing three different forms of PGE (see Fig. 1), that each developed a different mechanism for eliminating the paternal genomes (see Fig. 2). To address this, Herrick & Seger (1999) proposed that once PGE has evolved there would be strong selection on the paternally inherited genes to evolve a mechanism to resist being eliminated from the sperm. They argued that males could do this by either reversing the heterochromatinization of their chromosomes and having their chromosomes join the maternal euchromatinized set, or by resisting the disintegration of their paternal chromosomes and forming paternally derived sperm. They also stressed that even a mutation that would allow a small number of paternal genes to escape elimination would rapidly spread through the population and

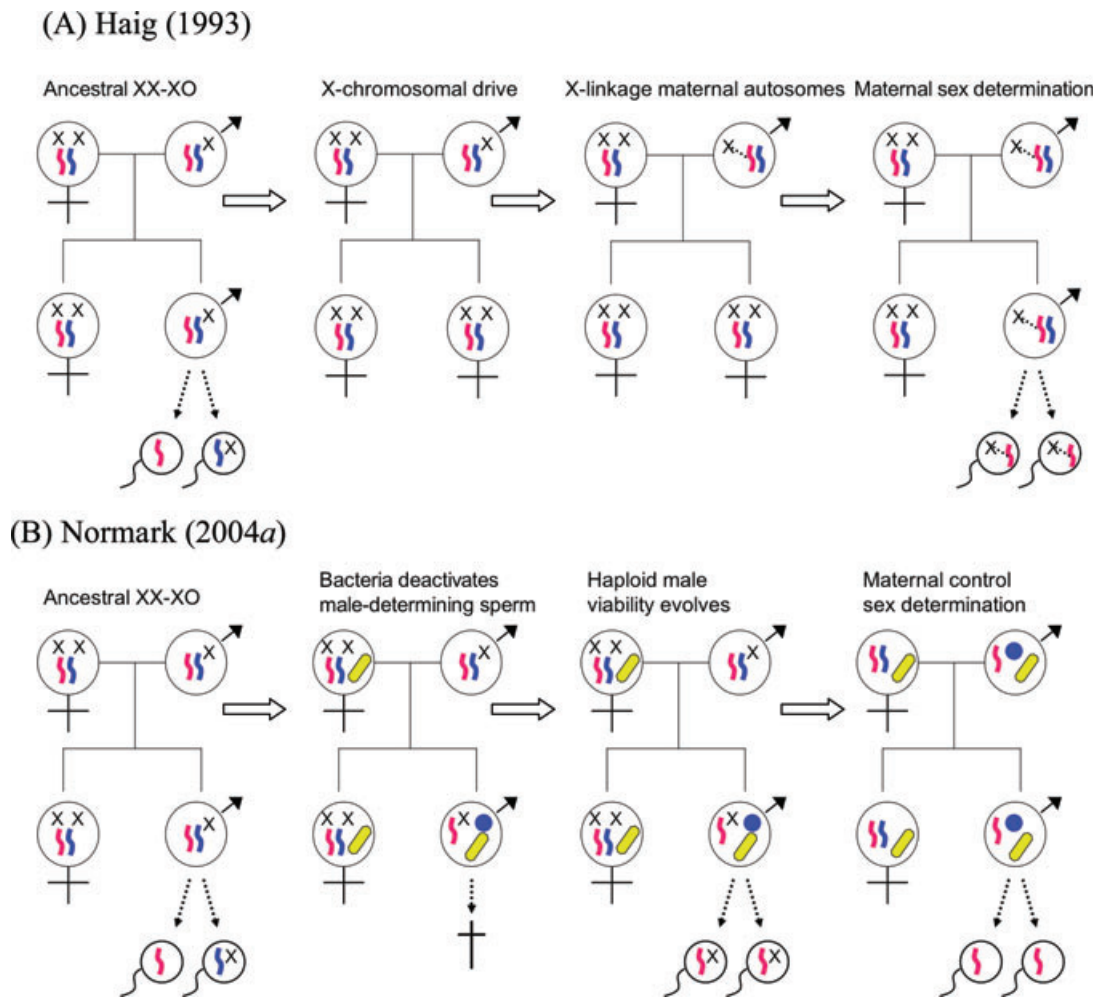


Fig. 4. A schematic representation of two hypotheses for the evolution of paternal genome elimination (PGE). In each the pink line represents the maternally inherited chromosome set while the blue line represents the paternally inherited chromosomes. The blue circle represents the heterochromatinized state of the paternally derived genome. The “X” represents the X-chromosome and the yellow oval represents the endosymbionts. (A) The X-chromosome drive hypothesis based on Haig (1993). (B) The male-killing endosymbionts hypothesis based on Normark (2004a).

lead to a strong selection pressure for maternally inherited genes to suppress the “leak”. They therefore suggested that this evolutionary arms race might have driven the evolution of earlier deactivation or elimination of the paternal genome in males and generated the variety of coccoid PGE systems (i.e. answering why there are several types of PGE and not just one).

Several other authors have proposed hypotheses for the variation in retention and loss of the paternal genome in species across the three different forms of PGE (Nur, 1980). Based on phylogenetic evidence, it has generally been assumed that the lecanoid system is the ancestral PGE system, with the Comstockiella and the Diaspidid systems being more derived (Fig. 1). Given this scenario, the earlier loss of the paternal genome has been explained by recourse to some (presumably metabolic) cost of retaining the inactive paternal genome (Nur, 1980). This hypothesis might be able to explain

the evolution of the Diaspidid system where the paternal genome is lost during early development and therefore any cost would be largely avoided. It is however hard to see how this could explain the evolution of the Comstockiella system, by far the most common genetic system in scale insects and an evolutionary transition that has taken place several times (Fig. 1). In the Comstockiella system, some paternal chromosomes are eliminated just before spermatogenesis. It is therefore hard to see how this could reduce the cost of the retention of the paternal genome to any great extent because the paternal genes are present in all cells in the soma and are only eliminated from the germline at a very late stage.

Herrick & Seger (1999) therefore proposed their hypothesis for the loss (or elimination) of paternal genes in the Comstockiella system, based on the observation that in some taxa with the lecanoid system of PGE paternal genes are reactivated during spermatogenesis. They proposed that the

timing of loss of paternal genes just before spermatogenesis is the result of antagonistic co-evolution between paternal and maternal genes over the extent of paternal gene expression during spermatogenesis, with maternal genes trying to avoid genetic conflict between the maternal and paternal genome over the elimination of the paternal genome during spermatogenesis. The transition between the lecanoid and Comstockiella systems seems to have evolved several times in both directions (Nur, 1980), which is suggestive (but not conclusive) evidence for co-evolutionary dynamics among paternal and maternal genes for control of spermatogenesis.

Additionally, Brown (1967) pointed out that the instability of the Eriococcidae (felt scales) chromosome systems, which have oscillated back and forth between Comstockiella and lecanoid systems for 80 million years, seems hard to reconcile with the great antiquity of the family. Again, co-evolution between the paternal and maternal genes over the early elimination or reactivation of the paternal genome might help explain these patterns. If the earlier elimination of paternal chromosomes can be explained by genetic conflict over the elimination of the paternal genome during spermatogenesis, we would expect that in more ancestral systems paternal chromosomes might occasionally manage to make it into the sperm. This could be very hard to observe though, both because it might be relatively rare and because of a lack of helpful molecular tools for these species. However, Nur (1970) observed that if the paternal chromosomes in *Planococcus citri* manage to become euchromatinized during spermatogenesis, then they can escape destruction. He also observed in several mealybug species that occasionally sperm are produced that have one or more extra (presumably paternal) chromosomes (Herrick & Seger, 1999). Clearly there is scope therefore for paternal genome leakage, although the extent to which this leakage is “accidental” or the result of variation in paternal gene expression (and selectable) is unknown.

Although direct proof for the struggle for the elimination of the paternal genome between the sexes is lacking, data from several taxa are very suggestive and might indicate a co-evolutionary struggle for control over transmission between the maternal and paternal genome. Although they are deactivated in most tissues, Nur (1967) showed that in the mealybug *P. citri*, which has the lecanoid form of PGE, the male genome is reactivated in several tissues. Perhaps tellingly, one of these tissues is the cyst where spermatogenesis takes place and where the paternal genome gets eliminated from the gametes! This supports an earlier finding that males with a paternal genome set that had been damaged by radiation could survive, but were sterile (Nelson-Rees, 1962), suggesting a crucial role for the paternal genome in spermatogenesis. The exact function of the sperm cyst, which is a structure present in many insect taxa, remains unknown but it has been suggested to be involved in the imprinting of the genes in the gametes (Buglia & Ferraro, 2004). These data are intriguing as there should be no selective advantage for the paternal genome set to assist spermatogenesis, except

if in doing so it might be able to prevent its own elimination from the sperm.

The involvement of the paternal genome in spermatogenesis also seems to differ between closely related species. Although heterochromatinization in the sperm cyst cells is reversed in all mealybug species so far studied, the cells of the testis sheath (that contains the sperm bundles) lack a heterochromatinized set in *Pseudococcus obscurus* and *Phenacoccus gossypii*, but do contain a heterochromatinized set in *Planococcus citri*. Furthermore the testis sheath cells in *P. citri* are also characterized by endoreduplication of the euchromatinized (maternal) chromosome set, leading to cells that have multiple copies of the maternal set but only a single deactivated copy of the paternal chromosomes (Nur, 1966b). We can again speculate that this pattern might be caused by antagonistic co-evolution between the sexes, with the paternal genome trying to become activated to preserve its transmission and the maternal genome evolving suppression, possibly by using endoreduplication of maternal chromosomes in order to increase the expression of maternal genes.

More tangible evidence for direct suppression of paternal genes in males by the maternal chromosomes comes from the observation that in experimentally produced haploid male embryos or embryo's in which certain regions are made haploid, the paternal chromosomes at first undergo normal heterochromatinization, but become euchromatinized later in development, suggesting that the presence of the maternal genome set is needed to “suppress” paternal chromosomes (Brown & Nur, 1964; Nur, 1962b). Most likely the deletion of the maternal genome set does not have an effect on the initial heterochromatinization because early in development there is not any gene expression in the embryo (Sabour, 1972) and development is regulated by maternally derived gene products, possibly the recently characterized histone protein HP1 (Bongiorni *et al.*, 2007). The suppression of the paternal genome set might also be closely linked (mechanistically) to the deletion of the paternal chromosomes during spermatogenesis. As alluded to above, Nur (1970) observed that in some mealybug species treated with high doses of radiation, occasionally the paternal genome set became decondensed in the spermatocytes and that these spermatocytes could give rise to diploid sperm containing the paternal chromosomes. This shows that euchromatinized paternal chromosomes do not disintegrate (i.e. they are viable). These findings indicate a possible mechanism for the elimination of the paternal chromosomes in which the euchromatic maternal chromosomes produce a substance that is harmful for chromosomes in a heterochromatinized state, but leaves euchromatic chromosomes unharmed. If paternally inherited chromosomes manage to decondense therefore, they are then left viable and able to enter the germ-line.

The previously described hypotheses mainly focus on the evolution of PGE. However Normark (2006) described a new theory to explain not just the evolution of PGE but also the whole variety of genetic systems in scale insects (and other taxa). He argued that if (1) there is prolonged

association of kin groups (e.g. gregarious broods, maternal care and so on) and (2) a relatedness asymmetry within the kin group, with the offspring being more related through their maternal than their paternal genes (e.g. sharing the same mother but different fathers), then paternal genes are selected that make offspring more selfish (mediated, for instance, through patterns of genomic imprinting). Normark (2006) argued that the evolution of what he called asymmetric systems, in which only one parent transmits their genes, will suppress this possibly harmful conflict among offspring. This theory could account both for the evolution of several types of parthenogenesis and for all the systems in which only the female transmits her genes to the next generation and the offspring are clonal (promoting cooperation within the clutch). The evolution of both haplodiploidy and PGE will reduce the amount of conflict because males either lack, or have deactivated, paternal genes and therefore males are selected to be less selfish than in the ancestral XX-XO system. However, as we will discuss below, the evolution of these genetic systems by such a process may simply replace one set of genetic conflicts with another.

V. GENETIC CONFLICT, SEX DETERMINATION AND SEX ALLOCATION

(1) Theoretical considerations

In the previous section we considered how maternally and paternally inherited genes may come into evolutionary conflict over transmission (i.e. getting into gametes), and that this conflict may select for the evolution of alternative genetic systems. A possible side-effect of such conflicts could be the biasing of the population sex ratio. Here we turn our attention more directly to conflicts over sex ratio, and consider situations in which different genetic entities (males and females, parents and offspring) may be selected to favour different patterns of offspring sex allocation (including the numerical sex ratio). The rationale here is that if different entities come into conflict over sex allocation, then the sexes may each be selected to try to control sex allocation by taking over or influencing sex determination (Shuker, Moynihan & Ross, 2009). Conflict over sex determination could then lead to the evolution of alternative genetic mechanisms. We will begin by developing this rationale further, before reviewing what is known about sex determination in scale insects. However, central to considering the role of intra-genomic conflict over the sex ratio is determining what sex ratios are favoured by males and females (and their offspring), and whether or not sex allocation is fixed or facultative. We will therefore conclude with a review of sex allocation in scale insects, highlighting the potential scenarios where conflict may arise.

The scope for conflicts of interest over sex ratio has been long recognised [for instance by Burt & Trivers (2006) and Hamilton (1967)]. The conflicts may be rather direct, as is the case if there are selfish genetic elements actively

influencing the sex of offspring, such as driving X- or Y-chromosomes, supernumerary B chromosomes, or parasitic endosymbiotic organisms such as male-killing bacteria (2006; Werren & Beukeboom, 1998). The conflicts initiated by such selfish elements may be intra-genomic (as with driving sex chromosomes) or inter-genomic (as with male-killers). Alternatively, the context for conflict over sex allocation may arise more indirectly. For instance, there can be conflict over the sex ratio between parents if one of the parents shares fewer copies of its genes with the offspring of one sex, as in asymmetric systems such as haplodiploidy, where haploid male offspring develop from unfertilized eggs, while females develop from fertilized eggs. In this situation, fathers favour a more female-biased sex ratio than the mother because they only share genes with their daughters, while mothers share genes with all their offspring (Hawkes, 1992; Trivers & Hare, 1976).

There can also be conflict between parents and offspring over the sex ratio (Trivers, 1974). For instance, in cases where parents produce adaptively skewed sex ratios because of processes such as local resource competition (LRC) or local mate competition (LMC) (Clark, 1978; Hamilton, 1967), large asymmetries in reproductive value can be created between the two offspring sexes, with the rare sex having a much higher reproductive value. This means that if the offspring could influence which sex they developed into, they would prefer to be the rarer sex. This has the consequence that under certain conditions the offspring prefer a less biased sex ratio than the parents (Trivers, 1974; Werren & Hatcher, 2000; but see Pen, 2006 and Trivers & Hare, 1976). Finally, other apparently mutualistic associations may still form the context for conflicts over sex ratio, for instance if the mutualistic benefits are provided more by one sex than another. This could indeed be a possibility in species of aphids or scale insects tended by ants, in which the females of the plant-feeder provide the honeydew resource the ants receive in payment for protection from arthropod predators. In this situation, the ants prefer a female bias in aphid or scale insect sex ratios, as they get greater benefits from larger symbiont herds.

Although the general theoretical framework for such genomic conflicts over sex ratio is reasonably well developed, there is a lack of direct evidence for their importance. Several models have shown that there should often be conflicts of interest over the sex ratio of the offspring, but outside of the social insects, there are currently few empirical tests of the assumptions of the models (thus confirming the conditions necessary for the conflict to be initiated) or specific tests of the models' outcomes (Shuker *et al.*, 2009). There might be several reasons for this lack of experimental confirmation. First, conflicts may be obscured, such that researchers have not really been encouraged to look for them. For example, it is possible that there are ongoing battles over the sex ratio but, because of coevolutionary processes in the past, the current conflict is hard to identify (Chapman, 2005; Kozielska *et al.*, 2009). In addition, although there might be a conflict of interest over sex ratio between different

parties (for example between the father and mother), not all parties may have the power to influence the sex ratio, as was assumed to be the case for haplodiploid Hymenoptera (Trivers & Hare, 1976; Werren & Beukeboom, 1998; but see Shuker *et al.*, 2006). Conflict might also have happened in the past but have been resolved by the evolution of a new genetic or sex-determination system. For all of these reasons it may be difficult to observe past or present conflicts over sex allocation.

(2) Sex determination in scale insects

What evidence is there that genomic conflict over sex allocation has led to the evolution of sex-determination mechanisms, and that genetic systems evolved along with sex determination? First, let us consider patterns of sex determination in scale insects. Basal scale insects have a XX-XO genetic system (Fig. 1B), which suggests that they have genetic sex determination. This is supported by the observation that in species of the genus *Puto* (a genus that was placed with the mealybugs previously but is now considered to be part of a separate family, the Putoidae) during spermatogenesis sperm both with and without an X chromosome are formed in equal numbers (Brown & Cleveland, 1968). However, although the XX-XO system found in more basal scale insects suggests that genetic sex determination is the ancestral sex-determination system, data on the closely related aphids suggests differently. In aphids that seem to have an XX-XO genetic system, it has been discovered that all zygotes are initially XX females, but that some zygotes develop into males after one of the X chromosomes is randomly lost (Wilson, Sunnucks & Hales, 1997). Males only form viable sperm that carries the X chromosome and therefore autosomes that are not associated with the X chromosome are not transmitted to the next generation (Wilson *et al.*, 1997). This system shows some interesting similarities to the paternal genome loss system found in many more derived scale insects (Section II.3). However, although the cytology of many basal scale insects has been studied extensively (as reviewed by Hughes-Schrader, 1948) a system similar to that described in aphids has never been found. It is therefore more likely that ancestral scale insects indeed had genetic sex determination and that the aphid system evolved independently in that lineage.

In other diploid scale insects there is the intriguing suggestion that endosymbiotic bacteria are associated directly with sex determination. For example, *Stictococcus* species lack both heterochromatinization and sex chromosomes and both sexes are diploid. However, the endosymbionts, although present in females, are absent in males. Buchner (1965) therefore suggested that in *Stictococcus* spp. sex is determined by the bacteria, with eggs containing bacteria developing into females, and eggs without bacteria developing into males. The diploid system found in *Stictococcus* spp. has clearly evolved from a PGE lineage (Fig. 1). It might be significant in this instance that the loss of the endosymbionts in males coincides with the loss of heterochromatinization in males.

However, definitive evidence for a role of the endosymbiotic bacteria as the sex-determining agent is still lacking.

In systems with paternal genome elimination the sex-determination mechanism is still poorly understood. No sex chromosomes have ever been observed in scale insects with PGE and the presence of autosomal genetic sex determination loci is also unlikely (Brown & Nur, 1964). Therefore it has been assumed that sex is determined either by facultative imprinting (offspring sex depends on the way the gametes are imprinted by the parents) or by maternal effect proteins that are added to the eggs. The latter hypothesis is supported by the observation that in several taxa with PGE the eggs containing male or female embryos differ in colour and that this colour difference is already present before fertilization (Nur, 1989). However, since maternal proteins may presumably influence methylation/other imprints as well, the two mechanisms could be hard to separate.

Despite our uncertainty over the mechanisms of sex determination themselves in PGE species, the molecular mechanism of how paternal chromosomes are eliminated has recently been unravelled, perhaps offering clues about the sex-determination process. Central to the process is the difference in genomic imprinting between the paternally and maternally inherited chromosomes. The paternal genome is hypomethylated in comparison to the maternal genome and this difference is present in both sexes of the species (Bongiorni, Cintio & Pranter, 1999). Recently, a histone protein has been identified that is responsible for the heterochromatinization of the paternal genome in males. This protein, a HP1 homolog, is preferentially attached to the paternal genome and seems to recruit other histone proteins to the complex. Using RNA interference (RNAi), Bongiorni *et al.* (2007) knocked out expression of the HP1 homolog and this resulted in a reversal of heterochromatinization in males and also a lack of recruitment of two other histone proteins. One remaining question is whether this protein is of maternal origin or is expressed by the embryo itself. Bongiorni *et al.* (2007) showed that cleavage-stage embryos were particularly sensitive to the RNAi treatment while treatment of more advanced embryos had little effect. Sabour (1972) showed that, in mealybugs, gene expression only occurs after the 5th division; by that time the paternal genome is already condensed in males. These findings suggest that the HP1 protein enabling heterochromatinization in males is indeed of maternal origin, suggesting both maternal influence over sex determination and providing a candidate for the maternal effect protein responsible for the control.

In a different vein however, Buglia & Ferraro (2004) recently suggested a paternal sex-determination mechanism in the mealybug *Planococcus citri*. They observed that during spermatogenesis some sperm cells carry a higher concentration of two histone proteins, HP1 and H3 analogs, discussed above as being involved in the heterochromatinization process in *P. citri* (Bongiorni *et al.*, 2007). They argue that the percentage of sperm “tagged” with these proteins (approximately 50%) is similar to the offspring sex ratio observed

in *P. citri*. They also hypothesised that the unusual mechanism of spermatogenesis found in coccoids, where sperm are formed in a sperm cyst, enables males to imprint their sperm in such a way as to influence the sex of the developing embryo. Although their findings are interesting they do not prove a direct relationship between the identified protein and sex determination, and it is also difficult to see how the paternal sex determination that they propose could have evolved as males are not expected to favour male production (see below).

Another suggestion for the sex-determination mechanism in PGE lineages comes from Buchner (1965) who, after observing the apparent involvement of endosymbionts in sex determination in *Stictococcus* spp. (see above), argued that endosymbionts might also be involved in PGE species. He envisaged bacterial titre being the key determinant, with eggs containing many bacteria developing into females, whilst eggs with low bacterial counts develop into males. This mechanism is supported by the observation that in the mealybug *P. citri* both high rearing temperatures and aging of females result in a reduction in endosymbionts and a more male-biased sex ratio (Brown & Bennett, 1957; Buchner, 1965; Kono *et al.*, 2008; Nelson-Rees, 1960). However, these data are circumstantial and do not exclude other correlated effects, either with respect to the bacteria or the scale insects themselves.

(3) Sex allocation patterns in scale insects

The extent to which genes will experience conflicting selection pressures relating to the genetic context they find themselves in (mothers or fathers, parents or offspring etc.) may depend on the sex ratios produced. For instance, if sex ratio selection has favoured female-biased sex ratios (to reduce local mate competition for example), then there may be rather little conflict over sex ratio between mothers and fathers in haplodiploid or PGE species (Shuker *et al.*, 2006). If sex ratio selection favours a male bias, there may be considerable conflict over sex ratio, as genes in fathers would suffer extremely reduced transmission compared to genes in mothers. The same will be true for parent-offspring conflicts over sex ratio, where sex ratio selection helps define the difference in sex ratio optima (Pen, 2006; Uller *et al.*, 2007). Here we consider the known variation in sex allocation in scale insects in order to identify the possible scope for sex ratio conflict. Unfortunately, although sex allocation data are available for several scale insect species, well-controlled experiments are limited and therefore reliable data are only available for a small number of species. We will give a short review of the sex ratio data available for coccoids, focussing especially on the few well-studied species and on those that seem to show a strong sex ratio bias.

Sex allocation in mealybugs has been studied most extensively in the mealybug *Planococcus citri* (Fig. 3E). Most studies show equal or slightly female-biased sex ratios (Nelson-Rees, 1960; Ross *et al.*, 2010; Varndell & Godfray, 1996). Several factors have been identified that affect the sex allocation of *P. citri* (as reviewed by Ross *et al.*, 2010). First of all, population density seems to affect the sex ratio,

although the effects are complex (Ross *et al.*, 2010; Varndell & Godfray, 1996). Second, temperature strongly affects sex ratio (James, 1937; Nelson-Rees, 1960) and finally the age of the female both at the time of mating and egg laying, affects sex allocation (Nelson-Rees, 1960; Ross *et al.*, 2010).

Sex allocation of armoured scales (Diaspididae) has been reviewed by Nur (1989). In general, most taxa produce 50:50 sex ratios. Interestingly though, in some species significantly male-biased sex ratios are observed. One of the best-studied armoured scale insects is *Pseudaulacaspis pentagona*; in this species there is a sexual dimorphism in the eggs, with coral red eggs containing female embryos and white eggs containing male embryos. Brown & Bennett (1957) showed that the age of a female strongly affected the sex ratio of her offspring, with a female producing only female offspring in the first few days of oviposition before switching to producing only males. They also showed that females that were prevented from mating for 20 days produced a more male-biased sex ratio once allowed to mate (Brown & Bennett, 1957). A similar effect has also been observed in two other Diaspidid species (Nur, 1989).

The best data on sex allocation patterns in the field comes from the work of Alstad & Edmunds (1983, 1989) on the black pineleaf scale (*Dynaspidiotus californicus*, as *Nuculaspis californica*, Fig. 3C). They studied sex ratio patterns in the field over several years across several different locations. They initially observed an extremely female-biased sex ratio in the adult population (less than 10% males; Alstad & Edmunds, 1983). Further studies however established that the sex ratio at the crawler stage was only slightly female biased (40 % males); the primary sex ratio remains unknown (Alstad & Edmunds, 1989), suggesting male-biased mortality.

Several authors have attempted to understand the sex allocation patterns in scale insects based on sex allocation theory, for instance the sex ratios predicted by local resource competition (LRC) theory (Hamilton, 1967; Ross *et al.*, 2010; Varndell & Godfray, 1996). The rationale here is that since female scale insects have a sedentary life style and form large colonies, related females (e.g. sisters) are likely to compete locally for resources, while males are able to disperse away from competition. LRC predicts male-biased sex ratios in order to reduce this local competition between related offspring. Recently it was shown that density affects sex allocation in *P. citri*, with females producing a more male-biased sex ratio under high densities, although density in that experiment reduced the extent of competitive interactions between kin relative to interactions among non-kin (Ross *et al.*, 2010). These results therefore did not support LRC theory but instead suggest that competition between unrelated individuals might affect sex allocation in this species (by reducing the reproductive value of daughters when “global” resource competition is high).

Many scale insects have a genetic system with asymmetric transmission and there is therefore a wide scope for conflicts over sex allocation in many taxa. Both in species with haplodiploidy and PGE, males “prefer” a more female-biased offspring sex ratio than their partner (as outlined above). The important question remains however as to whether or

not males have the power to influence sex determination. Although female control of offspring sex ratio has been observed in several species, there is the suggestion of male involvement as well. It will be very important to test this formally both by using within-generation experimental crosses and across-generation quantitative genetics experiments to estimate the amount of variation in sex ratio that can be attributed to male mating partner/genotype and by directly manipulating the mechanism with which males are suggested to influence the sex ratio, for example by using RNAi techniques to block HP1, a technique that has already been successfully applied when studying heterochromatinization in the embryos (Section V.2).

VI. INTER-GENOMIC CONFLICT: HOST-SYMBIONT CONFLICTS

In this section we will focus on genetic conflicts between scale insect hosts and their endosymbionts. We will both explore empirical data that suggest the possible involvement of the endosymbionts in the evolution of the different genetic systems and focus on conflict over sex determination and sex allocation between host and endosymbionts. We will begin by introducing the biology of the scale insect endosymbiosis.

(1) Endosymbiosis

Most scale insects have an obligate symbiotic relationship with one or more species of bacteria. Scale insects rely on their endosymbionts to synthesize and provide the essential amino acids and vitamins that are absent from their diet (Buchner, 1965; Fink, 1952). The endosymbiosis in scale insects and other plant-feeding insects has been well studied, especially by Buchner (1965), who summarised his findings in an extensive monograph. The relationships between scale insects and their symbionts are often ancient and many bacteria show strong patterns of co-speciation with their host (Baumann & Baumann, 2005; Gruwell, Morse & Normark, 2007). The endosymbiotic bacteria found in scale insects belong either to the Flavobacteria (Eriococcidae, Margarodidae, and Diaspididae) or to the Proteobacteria (Pseudococcidae, Putoidae, and possibly some Coccidae) (Gruwell *et al.*, 2004; von Dohlen *et al.*, 2001). However, the endosymbionts found in many soft scales (Coccidae) and in isolated members of other families are eukaryotes (fungi) (Buchner, 1965; Tremblay, 1989).

Endosymbiotic bacteria are typically confined to specialized organs (bacteriomes) that can make up to 30% of the body mass of the insect, although the structure and formation of these organs varies widely among different taxa (Tremblay, 1989). In mealybugs and armoured scale insects the bacteriome is formed by the fusion of the maternal polar bodies with embryonic cells (Normark, 2004b; Nur, 1990; Tremblay & Caltagirone, 1973). This results in a polyploid organ, which contains both the embryo's genome, as well as the three (maternally derived) polar body genomes.

The bacteria themselves are contained within the cytoplasm of the cells of the bacteriome (termed the bacteriocytes). In species of the family Putoidea the endosymbionts are actually transmitted within maternally derived bacteriocytes (Buchner, 1965). These bacteriocyte cells enter the oocytes, and during embryogenesis fuse with embryonic cells to form the bacteriocytes in the new embryo, transmitting both the endosymbionts as well as maternal genetic material. This results in a bacteriome of partly maternal origin and it also prevents the endosymbionts from ever coming into contact with offspring tissues. Soft scales, on the other hand, often lack a bacteriome and their endosymbionts float freely in the host haemolymph, and occasionally in modified polyploid fat cells. Curiously, as noted above, the endosymbionts in many soft scales are not bacteria but instead unicellular fungi (Buchner, 1965).

The various endosymbionts need to transmit themselves from one scale insect generation to another. As is typical for endosymbionts more generally (Buchner, 1965), they are vertically transmitted through the hosts' maternal line, *via* the cytoplasm of the eggs. As such, many groups have very specialized mechanisms to ensure as many eggs are infected as possible (Buchner, 1965; Tremblay, 1989). For example, in *Planococcus citri* individual bacteriocytes break loose from the bacteriome and fuse with the ovaries releasing their bacteria, which then travel towards the developing oocyte and penetrate it. Initially the bacteria stay at the anterior pole of the egg. After fertilization the new bacteriocytes start to form in the embryo by the fusion of the polar bodies and embryonic cells, before migrating in the direction of the endosymbionts. When the two meet the bacteriocytes absorb the endosymbionts, where they will effectively remain in culture before infecting the next generation of eggs (if in a female) or dying with or before the host (if in a male) (Schrader, 1922). Although the specialised bacteriocyte cells might allow the host to control the bacteria to some extent, during oogenesis there is a short period where the endosymbionts are not inside the cells and have free access to the host's haemolymph and thus may be in a position to manipulate host physiology. This might be important in terms of whether or not endosymbionts have the opportunity (*i.e.* the power) to influence reproductive processes such as sex determination.

Although most Sternorrhyncha harbour their bacteria in bacteriocytes, the mechanism of the formation of the bacteriome seems to have evolved independently several times. For example, different cell types, sometime maternal, sometimes embryonic, or sometimes both, give rise to the bacteriome. However, there seems to be one common characteristic of many bacteriomes, especially those in taxa with PGE (Normark, 2001), and that is that they often consist of polyploid cells (Buchner, 1965; Tremblay & Caltagirone, 1973). In order to understand better the function and the evolution of bacteriomes, it might be crucial to understand the function of this polyploidy.

In addition to the strong, obligate relationships with their primary endosymbionts, which are generally phylogenetically conserved within families (Gruwell *et al.*,

2007; Thao, Gullan & Baumann, 2002), some scale insects also have a whole range of secondary symbionts (Buchner, 1965). In many cases the secondary symbionts are less strongly associated with a particular host, with closely related scale insects sometimes harbouring very different secondary endosymbionts (Thao *et al.*, 2002). Compared to the primary endosymbionts, the function of the secondary endosymbionts is much less well understood. It might be that they take over or complement some of the tasks of the primary symbiont, or provide their host with other advantages such as facilitating host adaptation or disease resistance, as found in various species of aphid (Scarborough, Ferrari & Godfray, 2005; Tsuchida, 2004). However, it might also be possible that they are purely reproductive parasites that make use of the transmission apparatus associated with the primary endosymbiont without providing any of the benefits. In many species, the primary and secondary endosymbionts live in close proximity, often within the same host cell, and in mealybugs the secondary endosymbionts actually live inside the primary endosymbionts (von Dohlen *et al.*, 2001). The fact that in mealybugs the two bacteria have never been observed independently suggests a strong mutualism between the two, although this has not been formally established. Interestingly, although most scale insects have more than one endosymbiont, in soft scales no additional bacterial endosymbionts have been observed co-infecting alongside their yeast-like primary endosymbionts (but see Gruwell *et al.*, 2004).

(2) Conflicts over sex allocation

Host-endosymbiont conflict over sex allocation has been extensively studied in many taxa and is described in several reviews (Hurst, 1991; Werren, Nur & Wu, 1988). Until now however it has mainly focussed on a few well-known reproductive parasites mainly of the genera *Wolbachia* and *Cardinium* (Weeks, Tracy Reynolds & Hoffmann, 2002; Weeks, Velten & Stouthamer, 2003; Werren, 1997; Werren, Baldo & Clark, 2008). Very little is known however about conflict over sex allocation between hosts and their obligate mutualistic bacteria. Like many of the reproductive parasites, obligate mutualistic bacteria such as those found in most scale insects are strictly vertically transferred through the female line (Buchner, 1965). Therefore males do not transmit bacteria whilst females do. This results in the potential for conflict between the host and the bacteria over the sex ratio, with bacteria favouring a more female-biased sex ratio than the host, even to the point of the total eradication of males. Interestingly, such a conflict may mean that the interests of the endosymbionts are often aligned with the interests of genes in male scale insects under genetic systems such as PGE, which also favour female-biased offspring sex ratios as paternal genes are also only transmitted through female offspring.

The extent to which these conflicts should lead to or result in the evolution of alternative sex determination and genetic systems has been addressed to some extent. Reproductive parasites have evolved a whole array of mechanisms to affect their host's reproduction (Charlat, Hurst & Mercot, 2003; Werren, 1997), including feminization of genetic

males (Rigaud, 1997), male-killing (Hurst, 1991) and parthenogenesis induction (Stouthamer, Luck & Hamilton, 1990). In doing so, these parasites have often subverted the existing mechanisms of sex determination and made aspects of the genetic system redundant (for instance *via* the elimination of males). More specifically in terms of scale insects, Normark (2004a) has combined many aspects of their biology (and indeed other taxa with PGE or haplodiploidy) to try to explain the evolution of PGE and haplodiploidy. He pointed out that a determining feature of species in which these genetic systems have evolved is that they typically: (1) all have endosymbiotic bacteria; (2) all have gregarious broods leading to high levels of competition between siblings. In his model, Normark (2004a) showed that under these conditions the endosymbiotic bacteria are selected to evolve male-killing and he proposed that they could accomplish this by the deactivation of male-determining sperm, haploidizing the male embryo and thereby killing it. This would initially be detrimental to the host and there would therefore be strong selection for the evolution of haploid embryo viability. Once this had evolved, females that produced these haploid males would have a selective advantage, as their sons would transmit their genes at a higher rate [the premise of Brown (1964) and Bull (1979), as discussed above] (see Fig. 4B). Normark's (2004a) original model has since been tested and adapted by several authors (Engelstadter & Hurst, 2006; Kuijper & Pen, 2010; Ubeda & Normark, 2006). In the latter case, Kuijper & Pen (2010) have recently shown that, although the stable evolution of PGE and haplodiploidy in the original model was rare, it can evolve more easily with a subdivided, highly inbred population and when the endosymbionts are mutualistic.

In order to consider the possible role of endosymbionts in the evolution of genetic systems in scale insects, we will start by discussing the possible presence of these bacterial-induced phenotypes in scale insects and then discuss which bacteria might be responsible.

(3) Male-killing

Endosymbiotic bacteria are selected to have a male-killing phenotype when killing males will increase the fitness of related females, as this will benefit the fitness of the bacteria's relatives in those females (a kin selection benefit: Hurst, 1991). This will occur in situations where broods are gregarious and male and female offspring develop together and compete for resources; this situation is present in many scale insects where nymphs compete for space and resources both within the maternal structure they are raised in (e.g. ovisac, marsupium) and possibly also on their host plant (Normark, 2004a). However male-killing has not been observed in scale insects (but see below), although this might be caused by the fact that a shortage of males will often be hard to observe due to their small size and short lifespan. In most taxa where male-killing is observed the male-killing is active and usually occurs during early development (Hurst, 1991). In scale insects there could also be "passive" (or "incidental") male killing, where the bacteria simply do not function well in the male and

thereby cause increased mortality indirectly. Interestingly, Gruwell *et al.* (2004) found in their recent phylogeny of scale insect endosymbionts that the endosymbionts of several scale insect families are closely related to bacteria that are known to have a male-killing phenotype in ladybird beetles and cause parthenogenesis in other taxa. As such, the lack of male-killing in scale insects is perhaps unlikely to be due to phylogenetic constraint on the part of the endosymbionts.

Whilst there is no direct evidence for active early male-killing in scale insects, there are some suggestive data in the literature. In the black pineleaf scale (*Dynaspidiotus californicus*) extremely female-biased sex ratios were observed in the adult population (less than 10% males) (Alstad & Edmunds, 1983). As discussed above (Section V.3), it was later established that the sex ratio at the crawler stage was only slightly female biased (40% males), with the primary sex ratio remaining unknown (Alstad & Edmunds, 1989). These data therefore suggest massive male-biased mortality. Interestingly, the observed male mortality occurs relatively late in development compared to the male mortality observed in many species infected with male-killing bacteria, which would support the idea of more passive male-killing where the bacteria just do not work as effectively in males as they do in females. Unfortunately, nobody has tested directly the hypothesis that the extreme male mortality is caused by a bacterium with a male-killing phenotype. Another interesting observation is the behavioural difference between male and female nymphs of many armoured scale insects, where male crawlers feed on sites of the host plants that are both more nutritious, but also more exposed and dangerous than the sites chosen by their sisters (Normark, 2004a), thereby reducing competition with their sisters. This would be in the interest of the bacteria and it could be that males are forced to feed on these places as their bacteria do not provide them with enough nutrients. Unfortunately no experiments have yet been conducted to test this hypothesis.

Male-killing can have significant effects on host populations, influencing the evolution of mating systems and even leading to extinction (Dyson & Hurst, 2004). As such, co-evolutionary responses by the hosts are predicted. For instance, the rapid evolution of a zygotic male-killing suppressor has recently been observed in a species of butterfly (Hornett *et al.*, 2006). However, we suggest that scale insects might have evolved different ways to suppress male-killers. First of all, scale insect males might have evolved to compete as little as possible with their sisters, and as a consequence reduce the evolutionary benefit for the bacteria to express a male-killing phenotype. This might explain why males often stop feeding early in development and also why in some species males feed on different areas of the plants (see above). Second, male-killing can be avoided if the sex of the offspring is hidden from the bacteria. Normark (2004b) suggested that the peculiar formation of the bacteriome in armoured scales (the fusion of polar bodies with embryonic cells and some activity of the paternal genome) assures that the bacteria in both sexes are contained in similar tissues, this potentially being a mechanism to hide the sex of their host from the

bacteria. A third mechanism that could stop male-killing is to avoid the transmission of the endosymbionts to males in the first place. This is exactly what has happened in the genus *Stictococcus*, where the absence of the endosymbionts in males is compensated for by their mothers by the evolution of a placenta-like structure in order to feed their sons (Buchner, 1965). Decreasing the dependency of males on the endosymbionts might also help explain the evolution of the male life history of many scale insects, where males stop feeding and start losing their bacterial load at the same time as they start gonadal and somatic differentiation from females (Kono *et al.*, 2008) and therefore when they might be unable to “hide” their sex any longer.

(4) Parthenogenesis induction

Parthenogenetic reproduction is common among scale insects and has evolved many times among the different families and there are several different parthenogenetic systems (Nur, 1971). Although the evolution of parthenogenesis in scale insects is poorly understood, there is evidence for the involvement of endosymbiotic bacteria in several taxa.

Nur (1972) found that in a species of soft scale (*Parthenolecanium corni*) some females produced offspring by diploid arrhenotoky: females are produced sexually, but males develop from unfertilized eggs in which diploidy is restored, but have one chromosome set heterochromatinized (see transition 5, in Fig. 1), while others produced offspring by obligate automictic thelytoky (see transition 10). He observed that the asexually reproducing females contained needle-like bacteria in addition to the yeast-like endosymbionts normally found in soft scales. These bacteria were found in several tissues and not just in the fat cells like the fungi. He also observed that the bacteria had imperfect transmission, with transmission varying between females, such that between 20–90% of the embryos received the bacteria. The fact that this bacterium is not found in a specialized host structure, does not have perfect transmission, and is only found in part of the population, suggests that it is probably a reproductive parasite rather than an endosymbiont that benefits the host. It is also possible that it is in fact one of the known reproductive parasites previously identified in insects (such as a *Wolbachia* sp. or *Cardinium* sp.), although to date the identity of the bacterium has not been established. This was the first occasion that the presence of endosymbiotic bacteria was linked to asexual reproduction (Hurst, Godfray & Harvey, 1990), but unfortunately the theoretical framework needed to understand this finding had not been developed, and so the observation remained relatively unnoticed.

Recently the presence of the endosymbiotic bacteria *Cardinium* spp. has been confirmed in a number of species of armoured scale insects and the presence of *Cardinium* spp. has also been shown to coincide with several incidences of parthenogenesis (Gruwell, Wu & Normark, 2009). However, not all cases of parthenogenesis in armoured scale insects could be attributed to *Cardinium* spp. and currently there are no experimental data available showing a direct relationship (e.g. by removing *Cardinium* spp. with antibiotics).

(5) The role of host-endosymbiont conflict on the evolution of novel genetic systems

To date, empirical data supporting the role of host-endosymbiont conflict in the evolution of novel genetic systems is limited. Probably the best case for bacterial involvement in PGE systems comes from the observation that species of the genus *Stictococcus* that have lost the endosymbionts from males also lack the heterochromatinization of paternal chromosomes in males (Buchner, 1965). However, another case has been suggested by Royer (1975) based on his observations on *Icerya purchasi*. This hermaphroditic species contains both diploid germline cells, which produce oocytes and haploid germline cells that produce spermatozoa (see Section II.6). Royer (1975) noticed that the haploid germline cells always develop in close association with the endosymbiotic bacteria, which therefore might play a role in their development. The endosymbionts might perhaps therefore be involved in the origin of hermaphroditism in *I. purchasi*, which would be in their interest, as it results in a strongly female-biased sex ratio. There is also a suggestion that endosymbionts influence host reproduction in three species of the genus *Hippococcus* (*H. rappardi*, *H. wegeneri* and *H. montanus*). These species, which have an obligate relationship with ants, completely lack endosymbionts in both sexes. They do not seem to suffer from this lack of bacteria during their development, however adult females do not start forming oocytes while developing on the host plant, only doing so when the ants carry the adult females into their nest and feed them directly (Buchner, 1965). Perhaps some substances required for reproduction that are typically provided by the bacteria are in this case provided by the ant host. Several other taxa are known to have lost either their endosymbionts (in both, or just one sex) (Tremblay, 1989) or the heterochromatinization in males (Nur, 1980). It will be of great interest to focus on these taxa to see if there are more incidences of the absence of endosymbionts coinciding with a loss of heterochromatinization.

The role of endosymbionts in the evolution of parthenogenesis is well supported by several studies (see above), but all these examples are probably the result of reproductive parasites already discovered in other taxa. Moreover, only a small fraction of the known cases of parthenogenesis have been linked to endosymbionts. In particular, the presence of the wide variety of parthenogenetic systems in soft scale insects is not well understood. One possible hypothesis for the evolution of parthenogenesis in this group comes from Normark (2004b), given that the peculiar formation of the bacteriomes in many other scale insects (the fusion of polar body and embryo genomes) may serve as a way to hide the sex of the host from the bacteria. In many soft scale insects the endosymbionts are not contained in a specialized organ, but float freely in the host's haemolymph and even enter germ line cells (Buchner, 1965). It could be that this gives the host less control over the endosymbionts and that the actions of their endosymbionts could explain the evolution

of the more extraordinary systems observed in this group (including parthenogenesis).

Although there are suggestions that conflict between the scale insect host and their obligate endosymbionts might have affected the evolution of many aspects of scale insect biology, much of the data supporting these ideas are often not more than anecdotal observations on single taxa. In order to better understand how important host-symbiont conflict has been in shaping the evolution of scale insects we need a proper comparative test of the hypotheses outlined above, based on data from a wide range of species and placed firmly in a phylogenetic context.

VII. ANOTHER GENETIC CONFLICT IN COCCOIDS

Finally, we will briefly mention one last additional genetic element in scale insects that could potentially be an interested party in sex allocation: B chromosomes. B chromosomes are relatively common in Sternorrhyncha (Maryanska-Nadachowska, 2004), and in scale insects they are found to occur in at least three species, all of which have a PGE genetic system (Nur, 1962a; Nur, Brown & Beardsley, 1987). B chromosomes in scale insects were first observed by Nur (1962a) in the mealybug *Pseudococcus viburni* (previously *P. obscura*). He observed the presence of supernumerary chromosomes that behaved in a different way from the other chromosomes. *P. viburni* has a lecanoid PGE system, so in males the paternal chromosomes are silenced and not transmitted to the next generation. He observed, however, that certain supernumerary chromosomes when paternally derived behaved just like the other paternal chromosomes during development, but became euchromatic during spermatogenesis and segregated with the other maternally derived euchromatic chromosomes, thereby avoiding destruction (Nur, 1966a). Nur (1966a) also observed a strange behaviour of these chromosomes during oogenesis; in this circumstance they seemed to be preferentially excluded from the egg. This means that the B chromosomes are able to spread through the male line but are removed from the female line (in opposition to most reproductive parasites). Therefore B chromosomes in mealybugs are selected to favour male-biased sex ratios. In other families of Sternorrhyncha there is also the suggestion of involvement of B chromosomes in the evolution of genetic systems, particularly the evolution of sex chromosomes (Carvalho, 2002; Maryanska-Nadachowska, 2004). Apart from having a potential interest in sex allocation, the presence of B chromosomes and the mechanism by which they spread is extremely pertinent for PGE systems, as they represent the first evidence that it is possible for paternal chromosomes to avoid destruction during spermatogenesis. By studying the evolution of B chromosomes in species with PGE, we may get a more complete understanding about the conflict between the maternal and paternal genomes over the suppression and the deletion of the paternal chromosomes.

VIII. FURTHER DIRECTIONS

In this review we have attempted to highlight the extraordinary diversity of genetic systems in scale insects and outline the various different hypotheses put forward to explain them, with particular reference to the upsurge in interest in genomic conflict. However, as will have been apparent, we have been able to reveal lots of startling biology, and plenty of plausible hypotheses, but rather few robust attempts to link theory with empirical data, and little that could be called a compelling test of a given theory. We believe, though, that scale insects do have the potential to provide an exceptional resource for testing theories of genomic conflict, sex determination and the evolution of genetic systems. We would therefore like to finish with a short overview of experimental and comparative approaches that we believe will help us to understand the role of genomic conflict in shaping the biology of scale insects.

(1) Experimental and comparative approaches

Many of the hypotheses stated herein have not been formally tested. First it will be important to choose the right taxon to work with, depending on the particular question and hypothesis. To study the possible involvement of endosymbionts on the evolution of novel genetic systems and their role in sex determination, it will be best to focus on taxa that have lost or recently replaced their endosymbionts. An obvious choice here would be to focus on the African genus *Stictococcus*, in which only females have endosymbionts and in which there is a great deal of variation in endosymbiont status among closely related species (with some harbouring bacteria, others fungi and some both). To study sexual conflict over paternal genome elimination and sex allocation it will probably be best to focus on species with the ancestral lecanoid PGE system as paternal genes in this system might have more opportunity to influence transmission and sex allocation than in the other systems, as they are eliminated later in development. The obvious choice here would be the mealybugs, and especially the citrus mealybug *Planococcus citri*, as the pioneering work on both the genetic system and more recent work on the molecular mechanisms of PGE was carried out in this species.

One common pattern that is emerging is that the tissues that are expected to be the battlegrounds of both inter- and intra-genetic conflict (e.g. the bacteriome and parts of the testis) are characterized by both the reactivation of the paternal genome and by polyploidy. Furthermore, although this pattern seems widespread among species with PGE, there is a great deal of variation among closely related species in exactly which tissues the paternal genome is suppressed and in which the maternal genome becomes polyploid, suggesting the possible role of co-evolution between the sexes (in tissues involved in spermatogenesis) and between maternal, paternal and bacterial genes (in the bacteriome) as a driving force behind this variation. It might be possible, for instance, to test for maternal-paternal conflict over paternal genome

elimination by making hybrid crosses between closely related species that differ in their patterns of paternal gene expression to see if the paternal genes might be able to escape.

Another testable prediction is the possible role of the endosymbiont in its host's sex determination. This could be done by manipulation of the bacterial titre or by studying sex allocation of old females that might not be able to transfer as much bacteria to their eggs. If the bacteriome has a function in avoiding conflict between the host and its bacteria then one would expect more influence of the bacteria on sex determination in taxa that lack a bacteriome. One would also expect to find a difference between taxa in which the bacteria have a "free" phase during transmission and those where the bacteria are transmitted within maternal bacteriome cells (e.g. Putoidae). It might be possible to address this by a comparative analysis, linking the variability of genetic systems with the amount of time the endosymbionts spend outside the bacteriocytes.

If the earlier deletion of paternal chromosomes in the *Comstockiella* system functions to prevent paternal chromosomes escaping destruction during spermatogenesis, then one might expect that B chromosomes, which manage to do exactly that, will be more prevalent and successful in species with a lecanoid PGE system or a *Comstockiella* system in which relatively few chromosomes are destroyed before spermatogenesis. Unfortunately data on the prevalence of B chromosomes in scale insects are limited. It would therefore be valuable to screen for B chromosomes, especially in families that have species with both lecanoid and *Comstockiella* systems.

Finally, it might be possible to address some of the hypotheses discussed herein *via* comprehensive comparative analyses across scale insect taxa, using data on endosymbiont status, genetic system and other life-history traits combined with recently available phylogenetic data. We are sure such attempts will prove fascinating.

IX. CONCLUSIONS

(1) The broad array of diverse genetic systems in scale insects, and the multiple evolutionary transitions between them, provide an ideal opportunity to test theories regarding the evolution of genetic and sex-determination systems.

(2) Existing theories to explain this diversity in scale insects focus on either scale insect biology or the role of genetic conflict.

(3) Circumstantial evidence for the role of genetic conflict (within- and across-genomes) exists, but few compelling, independent tests of theory have been performed.

(4) With new phylogenetic information becoming available and increasing knowledge of some the mechanisms underpinning sex determination in scale insects, comparative analyses may provide the basis for these much-needed tests.

(5) Further study of scale insect biology is likely to yield fresh insight into the evolutionary significance of genetic conflict.

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