Genetic conflict and sex allocation in scale insects
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The role of endosymbionts in the evolution of haploid-male genetic systems in scale insects (Coccoidea)

Laura Ross, David M. Shuker, Benjamin B. Normark & Ido Pen

There is an extraordinary diversity in genetic systems across species, but this variation remains poorly understood. In part this is because the mechanisms responsible for transitions between systems are often unknown. A recent hypothesis has suggested that conflict between hosts and endosymbiotic micro-organisms over transmission could drive the transition from diplodiploidy to systems with male-haploidy (haplodiploidy, including arrhenotoky and paternal genome elimination). Here we present the first formal test of this idea with a comparative analysis across scale insects (Hemiptera:Coccoidea). Scale insects are renowned for their large variation in genetic systems, and multiple transitions between diplodiploidy and haplodiploidy have taken place within this group. Additionally most species rely on endosymbiotic micro-organisms to provide them with essential nutrients lacking in their diet. We show that species harbouring endosymbionts are indeed more likely to have a genetic system with male-haploidy, which supports the hypothesis that endosymbionts might have played a role in the transition to haplodiploidy. We also extended our analysis to consider the relationship between endosymbiont presence and transitions to parthenogenesis. Although in scale insects there is no such overall association, species harbouring eukaryote endosymbionts were more likely to be parthenogenetic than those with bacterial symbionts.

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INTRODUCTION

There is an extraordinary diversity in genetic systems across species, which includes variation in ploidy level, presence or absence of sexual reproduction and difference in sex determination mechanisms (Normark, 2003; Norton et al., 1993; White, 1973). However this variation is not spread equally across the tree of life: within some higher taxa there is no variation at all while in others variation exists between closely related species or indeed within a single species. One group that stands out for the extraordinary diversity of its genetic systems is the scale insects (Nur, 1980; Ross et al., 2010b). Scale insects comprise one superfamily within the order Hemiptera, yet there is almost as much variation in genetic systems within scale insects (see Table 6.1) as there is across insects as a whole (Normark, 2003). Recently ideas that intergenomic conflict can shape patterns of genetic system evolution have come to prominence (Bull, 1983; Burt & Trivers, 2006; Hamilton, 1993; Uller et al., 2007; Werren & Beukeboom, 1998), including in scale insects (Brown, 1964; Normark, 2004a, 2006; Ross et al., 2010b; Shuker et al., 2009).

In order to understand the variation in genetic systems we need to understand the transitions between the different systems. In scale insects it has generally been assumed that diplodiploidy with a genetic sex determination system (in this case XX-XO) is the ancestral genetic system (Nur, 1980). This system is found in most of the families within the relatively species-poor paraphyletic assemblage traditionally referred to as the Archaeococcoidea. However, the majority of scale insect species have a remarkable genetic system called paternal genome elimination (PGE). In this system both sexes develop from fertilized eggs and are diploid. However in males the chromosomes inherited from the father are deactivated during early development and subsequently lost from the germline during spermatogenesis (Brown & Nelson-Rees, 1961; Schrader, 1921). PGE is a synapomorphy of a major clade of scale insects, informally termed Neococcoidea. It has previously been suggested that since this remarkable system shows interesting similarities with arrhenotoky (where females develop from fertilized and males from unfertilized eggs) it might constitute an intermediate stage between diplodiploidy and arrhenotoky (Bull, 1979; Bull, 1983; Schrader & Hughes-Schrader, 1931). There is some evidence for this in mites (Cruickshank & Thomas, 1999), but in scale insects extant PGE and arrhenotokous clades have clearly evolved independently from diplodiploidy (Cook et al., 2002). Although diplodiploidy has been assumed to be ancestral, in a few taxa diplodiploidy seems to be a derived feature resulting from a reversion from PGE to diplodiploidy (Nur, 1980). These taxa, which generally lack sex chromosomes, are of particular interest when trying to understand the evolution of the variety of genetic systems in scale insects (Herrick & Seger, 1999; Ross et al., 2010b). Another important genetic system found in scale insects is hermaphroditism, a system found in no other insects (Hughes-Schrader, 1925; Normark, 2003; Royer, 1975). Apart from a variety of sexual reproductive systems, asexual reproduction is also common in scale insects, found in members of both the Archaeococcoidea and in the Neococcoidea. Again
there is a lot of variation in the form of asexuality, with up to six different systems described (Table 1: Nur, 1971; Ross et al., 2010b).

Several hypotheses have been brought forward to explain the transition between genetic systems and the resulting diversity in scale insects (as reviewed by Ross et al., 2010b). Although some of these hypotheses are plausible none of them have yet been formally tested. One recent hypothesis focuses on the transition from diplodiploidy to systems with haplodiploidy (arrhenotoky and PGE) and considers endosymbiotic bacteria the key driver of this transition through conflicts between hosts and endosymbionts over transmission (Normark, 2004a). We will first briefly review the presence and significance of endosymbionts in scale insects before detailing Normark’s hypothesis.

### Table 6.1 The genetic systems observed in scale insects (Normark, 2003; Nur, 1980; Ross et al., 2010b).

<table>
<thead>
<tr>
<th>Genetic system</th>
<th>Description</th>
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<tbody>
<tr>
<td><strong>SEXUAL SYSTEMS</strong></td>
<td></td>
</tr>
<tr>
<td>Diplodiploidy (XX-XO)</td>
<td>Both sexes develop from fertilized eggs and are diploid. Females are XX, males XO.</td>
</tr>
<tr>
<td>Diplodiploidy (2N-2N)</td>
<td>Both sexes develop from fertilized eggs and are diploid. No sex chromosomes have been observed</td>
</tr>
<tr>
<td>Arrhenotoky</td>
<td>Females develop from fertilized eggs and are diploid, males develop from unfertilized eggs and are haploid</td>
</tr>
<tr>
<td>Hermaphroditism</td>
<td>Diploid hermaphroditic individuals have a diploid female reproductive system producing oocytes and haploid testis cells producing sperm</td>
</tr>
<tr>
<td>Germline paternal genome elimination (lecanoid, Comstockiella,)</td>
<td>Both sexes develop from fertilized eggs and are diploid but in males paternal genes are deactivated during early development and subsequently not transmitted.</td>
</tr>
<tr>
<td>Embryonic paternal genome elimination (Diaspidid)</td>
<td>Both sexes develop from fertilized eggs and are diploid but in males paternal genes are lost during early development rendering males haploid.</td>
</tr>
<tr>
<td>Diploid arrhenotoky</td>
<td>Females develop from fertilized eggs and are diploid, males develop from unfertilized eggs, become diploid due to fusion of the haploid cleavage nuclei, but have haploid gene expression as one of the two genome sets is deactivated.</td>
</tr>
<tr>
<td><strong>ASEXUAL SYSTEMS</strong></td>
<td></td>
</tr>
<tr>
<td>Deuteroky</td>
<td>Reproduction can be both sexual and asexual and both males and females can develop from either fertilized or unfertilized eggs. Individuals that develop from unfertilized eggs restore diploidy by fusion of the first haploid cleavage nuclei.</td>
</tr>
<tr>
<td>Automicthic Thelytoky</td>
<td>Females develop from unfertilized eggs, males are absent. Meiosis is normal and diploidy is restored either by the fusion one polar body with the pronucleus, or by the fusion of the first haploid cleavage nuclei.</td>
</tr>
<tr>
<td>Apomicthic Thelytoky</td>
<td>Females develop from unfertilized eggs, males are absent. Meiosis does not take place</td>
</tr>
</tbody>
</table>
Scale insects, like many Hemiptera, feed almost exclusively on phloem of their host plant. This constitutes a problem, as phloem is very rich in sugars but poor in other nutrients, most notably in essential amino acids. In order to compensate for the imbalance in their diet, many phloem-feeding insect have engaged in a symbiotic relationship with micro-organisms (Buchner, 1965; Moran & Telang, 1998). It has even been suggested that this evolutionary invention has allowed them to colonize a niche that would have otherwise been out of reach and has allowed the rapid diversification of phloem feeders (Gullan & Kosztarab, 1997). Most scale insect species have an obligate relationship with one or several micro-organisms, which live inside the host cells (Buchner, 1965; Tremblay, 1989; Tremblay, 1997). The relationship between host and endosymbiont is often close, and several endosymbiont taxa have been found to have phylogenies that parallel those of their hosts, indicating strict vertical transmission (Baumann & Baumann, 2005; Downie & Gullan, 2005; Gruwell et al., 2007). Scale insect endosymbionts are transmitted through the female line and a variety of mechanisms have evolved to ensure successful transmission of symbionts from a mother to offspring (Buchner, 1965; Tremblay, 1989). Another feature which indicates the close association between host and endosymbiont is that in many species endosymbionts are kept in specialized cells (bacteriocytes) or even in a specialized organ (the bacteriome: Buchner, 1965).

Although obligate endosymbiosis is found across many Hemiptera, in scale insects the absence of endosymbionts is relatively common (at least compared to aphids and whiteflies: Buchner, 1965; Tremblay, 1989). There are several possible explanations for this. In a number of cases changes in diet or feeding behaviour are associated with the loss of endosymbionts. These include species that have switched to feeding on parenchyma tissues (Gullan & Kosztarab, 1997), species that form galls (Cook, in prep.) and species that are obligately associated with ants (three mealybugs of the genus Hippeococcus) (Buchner, 1965). Additionally in the Stictococcidae, males are fed by mothers via a placenta-like structure, do not feed independently, and lack endosymbionts during all life stages, though Stictococcid females do harbor endosymbionts. Within taxa that posses endosymbionts there is also variation in the type of endosymbiont (Buchner, 1965; Gruwell et al., 2004; Tremblay, 1989). The endosymbionts of most species are bacteria, but in several species the endosymbionts are unicellular fungi.

Normark’s (2004a) suggestion that the endosymbionts of scale insects might have played a role in the evolution of the observed variation in their genetic systems is based on the fact that endosymbionts are vertically transmitted, but only through the female line. This creates conflict between host and endosymbiont, as males constitute an evolutionary dead end for the symbionts. Endosymbionts are therefore selected to try to manipulate their host’s reproduction towards producing more female offspring. Several endosymbiotic bacteria have been found to manipulate host reproduction, most notably by inducing asexual reproduction, which removes the need for males altogether, thereby resolving the conflict between host and endosymbiont (Hurst et al., 1990; Stouthamer et al., 1990). Instead of manipulating host reproduction
directly, another way for endosymbionts to increase their inclusive fitness is by killing their host when they find themselves in a male. This might benefit the bacteria if there is competition between siblings and the resources that become available though the dead of a male can be utilized by its sisters, which carry bacteria that are related to those in killed males (Hurst, 1991). Male-killing phenotypes have indeed been observed in several endosymbionts (Hurst, 1991). Normark’s (2004a) hypothesis takes advantage of the fact that the life history of many species with haplodiploidy and PGE leads to strong and prolonged interactions between kin and that most of them contain endosymbiotic bacteria. Under these conditions male-killing may evolve, for instance with the endosymbionts destroying or deactivating incoming male-determining sperm. This would haploidize male offspring, and generally kill them. As a result, there would be strong selection for haploid viability of males, with any mutation responsible spreading rapidly as haploid males will always pass on this mutation (haploid transmission advantage).

One problem with Normark’s hypothesis is that male-killing phenotypes have only been observed for reproductive parasites that do not provide their hosts with any benefits, while the endosymbionts present in many haplodiploid and PGE species are obligate mutualists. Furthermore, whilst several additions to Normark’s original model have since been published, confirming the plausibility of Normark’s original hypothesis (Engelstadter & Hurst, 2006; Kuijper & Pen, 2010; Ubeda & Normark, 2006), these studies also point out that the scenario is more likely when the transmission efficiency is high, which would be expected to be the case for mutualistic endosymbionts but not necessarily for reproductive parasites.

Although Normark’s idea has received theoretical attention, no formal attempts have yet been made to try to test this hypothesis. Under Normark’s hypothesis we would expect that species that have endosymbionts are more likely to have male-haploid genetic systems compared to those that do not contain endosymbionts. Here we present results from a comparative analysis based on data from 582 scale insect species in 27 families. We first test if there is a relationship between the presence or absence of bacteria and their genetic system (diploidiploidy vs. male-haploid systems). Then we extend our treatment of the evolutionary significance of the association between endosymbionts and genetic system by considering a possible relationship between endosymbiont presence and asexual reproduction, including in this a role of endosymbiont identity (bacterial versus eukaryote). Finally we consider the importance of the intimacy of the relationship between host and the endosymbiont (i.e. specialised cells or tissues for the symbiont), as differences in how the host and endosymbiont interface inside the host may predict the extent to which endosymbionts can manipulate the host.
METHODS

Data collection
The data used for this analysis were collected between April 2007 and August 2009. We used a variety of sources. The main source for information on the genetic systems of scale insects was a recent review by Gavrilov (2007), which lists published information on genetic systems in scale insects. For information on endosymbiont status and identity the primary source was Buchner's (1965) extensive monograph on endosymbiosis and the references therein. Another important source was ScaleNet, an online database that collects an extensive amount of data on scale insect biology and is also an important source for literature on scale insects. In addition, new references were identified via Web of Science and Google Scholar as well as by inspecting the references of all papers of interest and by searching for citations of key papers. The taxonomy used in the analysis was based on the standard arrangement of families given in ScaleNet (Ben-Dov, Miller & Gibson, 2010), except that Margarodidae has been broken into several families per a recent revision (Hodgson & Foldi, 2006). This reflects the current standard classification used for example in Gullan and Cook (2007). Figure 1 shows the relationships between the scale insect families for which data were available in this analysis. For a few families no data were available for any of the considered factors. These are the Carayonemidae, Coelostomidiidae, Pityococcidae and Stigmacoccidae, (Archaeococcoidea) and the Micrococcidae (Neococcoidea). In species of the family Stictococcidae, only females harbor endosymbionts. Following the rationale of Normark -- that it is only the presence of endosymbionts in males that selects for male-killing -- we included the Stictococcidae as "endosymbionts absent".

Analysis
Phylogenetic inertia can cause statistical problems, as closely related taxa are more similar to one-another than more distantly related taxa are, thus violating the assumption of independence (Felsenstein, 1985). Therefore, in order to obtain reliable estimates from a comparative analysis, it is important to include information on the evolutionary relationships between the taxa included in the analysis. This information can come from taxonomy or from (molecular) phylogenetic inferences (Harvey & Pagel, 1991).

Several molecular phylogenies describing the relationships between scale insects have recently been published. Some of these studies focus on relationship between species within the different families: Diaspididae (Morse & Normark, 2006), Planococcidae (Downie & Gullan, 2004; Hardy et al., 2008), Eriococcidae (Cook & Gullan, 2004) and Monophlebiae (Unruh & Gullan, 2008). Other studies explore the relationships between families (Cook et al., 2002; Gullan & Cook, 2007). Although sequence data is available for more than 250 species, many of the relationships remain poorly resolved, especially at higher taxonomic levels.

The method adopted in this paper is to use a generalized linear mixed model
(GLMM) approach where the relationship between taxa can be fitted as a random effect. For a taxonomic GLMM, taxonomic classification (for example, order, family, genus) can be fitted to include species relationships while in a phylogenetic GLMM the phylogenetic relationship between species can be fitted as a random effect (based on branch lengths between nodes) (Hadfield & Nakagawa, 2010). The latter would be the preferred method, but reliable phylogenies are not always available. We chose not to use a species-level phylogeny partly because of the issues described above and partly because although sequence data is available for a reasonable number of scale insect species, there is little overlap with the species for which relevant data on genetic systems and endosymbionts are available. We included family as a random effect, but chose not to include genus, as there is little variation in genetic system or endosymbiont status within families (see results). As such, our analysis assumes a largely polytomic relationship between families. However, in order to include some information on the higher relationships between families, we divided the families into two groups, the Archaeococcoidea and the Neococcoidea. Although Archaeococcoidea is almost certainly paraphyletic, the Neococcoidea is one of the few well-supported supra-family groupings within scale insects (Gullan & Cook, 2007)(Figure 6.1).

We tested for a relationship between our characters of interest -- genetic system, reproductive mode (sexual vs. asexual reproduction), endosymbiont presence and identity and bacteriome presence (see Figure 6.2)-- using a bivariate binary mixed model approach. The models were fitted using the R package MCMCglmm (Hadfield, 2010a), which provides a Bayesian framework for generalized mixed model analysis. We used a multivariate normal prior for the fixed effects with a null mean vector and a diagonal covariance matrix with variances of $10+\pi 2/3$, which is approximately flat on the probability scale when the sum of the variance components is 10 (roughly the posterior mode). An inverse Wishart prior was used for the family covariance matrix, with the covariance matrix at the limit set to an identity matrix, and degree of belief parameter 1.002. This is equivalent to having marginal inverse gamma priors for each variance, with the scale and shape parameters set to 0.001 (Spiegelhalter et al., 2003). We also used a prior with the covariance matrix at the limit set to a diagonal matrix with the variances set to $10+\pi 2/3$, and degree of belief parameter set to 3. This is an approximately flat prior for the correlation coefficient. The residual variances were fixed at one because they are not identifiable in binary models, and the residual correlation was set to zero. The residual correlation is generally estimable in bivariate binary models, but in each of the different analyses performed very few families were variable for both traits considered, thus there is little information to estimate the within-family correlation. An additional model in which the residual correlation was estimated (with a weak prior; Barnard’s (2000) prior with degree of belief parameter equal to 5) was run for each analysis and gave qualitatively (and quantitatively) similar answers. The models were all run for 13 million iterations and the first 3 million iterations were discarded to ensure that the models had converged.

The analysis calculates the correlation coefficient between two response variables on the link scale at the family level. For simplicity, we will denote this correlation...
Figure 6.1 Schematic representation of the phylogenetic relationships between the Coccoid families included in our analysis. The solid lines are based on published phylogenetic studies (Cook & Gullan, 2004; Cook et al., 2002; Gullan & Cook, 2007)(Andersen in prep.; Normark, unpublished data), while the dashed lines show the hypothetical relationships of families for which no published sequence data are available, based mostly on a recent review of their taxonomic status (Gullan & Cook, 2007). Turquoise lines show the relationships between Archaeococcoids, while the black lines show the relationships between the Neococcoids. The pictures show slide-mounted specimens of representative species of each family.
coefficient as $r$. For each estimate we also calculate the 95% credibility interval (a Bayesian analogue to the confidence interval), which we will refer to as a 95% CI. We consider the correlation between two factors to be statistically significant if the 95% credibility estimates do not include zero. In order to test how much of the variation of a given response is explained by “family” we calculated the intraclass correlation (correlation between the estimated phenotype of two species within the same family) given by (Hadfield, 2010a), as a measure of phylogenetic signal:

$$\frac{\sigma^2_{\text{family}}}{\sigma^2_{\text{family}} + \sigma^2_{\text{residual}} + \pi^2/3}$$

In order to test if taxonomic group (Archaeococcoidea vs. Neococcoidea) had a significant effect on the different factors considered in this analysis we estimated the fixed effects (taxonomic group was fitted as a fixed effect, as it only has two levels), presented as the posterior mode and 95% CI.

RESULTS

The presence of diplodiploidy was strongly associated with the absence of endosymbionts $r = -0.96$ (95% CI = $-1.00 - -0.61$), $n = 432$ (Figure 6.2B). Family explained most of the variation for both the presence of endosymbionts and that of diplodiploidy. The intra-class correlation was $r = 0.72$ (95% CI = $0.36 - 0.95$) for the presence of diplodiploidy and $r = 0.97$ (95% CI = $0.83 - 0.99$) for presence of endosymbionts. There was also a significant difference between the Neococcoids and Archaeococcoids in the presence of diplodiploidy (fraction of species with diplodiploidy, Archaeococcoidea = 0.60, Neococcoidea = 0.008, posterior mode: $-8.89$, 95% CI = $-12.72 - -5.74$). However there was no significant difference between the two scale insect taxonomic groups in terms of the occurrence of endosymbionts (fraction of species with endosymbionts, Archaeococcoidea = 0.76, Neococcoidea = 0.89, posterior mode: 0.36, 95% CI = $-3.91 - 5.96$).

We also tested for a possible relationship between endosymbiont presence and reproductive mode (asexual versus sexual reproduction). First of all, there was much more within-family variation in reproductive mode than in the occurrence of diplodiploidy (the intraclass correlation for reproductive mode: $r = 0.35$, 95% CI = $0.10 - 0.55$). There was a negative correlation between endosymbiont presence and reproductive mode, however the effect is not significant ($r = -0.89$, 95% CI = $-0.98 - 0.17$, $n= 475$, Figure 6.2C). This suggests that endosymbiont presence does not significantly correlate with the presence of asexual reproduction. We also tested if the reproductive mode (sexual vs. asexual reproduction) was related to the identity of the endosymbiont. We found that there was a correlation between reproductive mode and bacteria identity, with asexuality being more common in species that contain eukaryote endosymbionts and this relationship was significant ($\hat{\rho} = -0.95$, 95% CI= $-0.99 - -0.23$, $n= 447$, Figure 5.2D).
Finally, we tested if the identity of endosymbionts was associated with how closely integrated the endosymbionts are by the host (i.e. in a specialized organ or not). We found that eukaryote endosymbionts were less likely to be housed within a bacteriome ($r = -0.97$, 95% CI = $-0.99$ – $-0.27$). The intra-class correlation of both factors was high, suggesting that family explains a large part of the variation in these factors (endosymbiont id: $r = 0.86$, 95% CI = 0.59 – 0.99, bacteriome: $r = 0.85$, 95% CI = 0.60 – 0.97, n = 238).
DISCUSSION

Scale insects that possess endosymbionts are more likely to have a haplodiploid genetic system. This supports the hypothesis postulated by Normark (2004a) who considered that coevolution between host and endosymbionts with a male-killing phenotype could have led to the evolution of male-haploid genetic systems. The results presented here are the first formal analysis that shows support for this hypothesis. Male-haploid genetic systems are found in a large number of taxa of both insects and mites. It will be of great interest to see if a similar role of endosymbionts can be observed in these systems.

Normark’s (2004a) hypothesis considers coevolution between male-killing endosymbionts and their host as the driving force behind the transition to male-haploid genetic systems. However, recently it has been shown that another phenotype commonly induced by endosymbionts, cytoplasmic incompatibility (CI), might also lead to conflict between host and symbiont resulting in the evolution of male-haploid genetic systems (Engelstadter & Hurst, 2006). Both mechanisms are consistent with our findings.

Although we find a relationship between genetic system and endosymbiont presence, we do not find a relationship with reproductive mode (i.e. sexual versus asexual). However the type of endosymbiont (bacteria or unicellular fungi) does correlate with reproductive mode, with asexual reproduction more often found in species with eukaryote endosymbionts. This could be because the latter are generally distributed freely in the haemolymph and are even found to be able to penetrate a variety of cells (including germline cells) (Tremblay, 1989). This means that they might be less tightly controlled by their host than bacterial endosymbionts, which generally are restricted to specialized cells, giving them more opportunity to influence their host’s reproduction (Ross et al., 2010b).

The role of endosymbionts in the evolution of asexual reproduction has been well established in many insects (Duron et al., 2008; Hurst et al., 1990; Koivisto & Braig, 2003; Stouthamer et al., 1990; Weeks et al., 2002; Weeks et al., 2003; Werren, 1997; Werren et al., 2008; Zchori-Fein & Perlman, 2004) and the endosymbiotic bacterium Cardinium is associated with parthenogenesis in a species of armoured scale insect (Provencher et al., 2005). The fact that in our analysis we do not find strong support for a generalised role of endosymbionts on the presence of asexual reproduction in scale insects might be due to the fact that in our analysis we simply used presence or absence, and were unable to distinguish between primary and secondary symbionts, as the role of the symbionts described in scale insects are mostly unknown. However, previous analyses have mainly found that the effect of secondary (often purely parasitic) bacteria is associated with asexual reproduction. More work on scale insect endosymbionts is clearly merited.

Apart from the presence of endosymbionts, population structures leading to high levels of kin competition is an important assumption of Normark’s model, as selection on the endosymbiont for a male-killing phenotype will only be strong under such
conditions. Scale insects usually have gregarious clutches and have evolved a variety of ways in which eggs and larvae are protected by the mother (in a marsupium, or ovisac for example). Additionally, crawlers often settle close to their mothers (Gullan & Kosztarab, 1997). All these factors lead to prolonged associations between kin and could therefore lead to high levels of kin competition (Normark, 2004a, 2006; Ross et al., 2010b). This might help to explain how endosymbiosis in scale insects could have led to conflict between endosymbiont and host, and resulted in a change in the host’s genetic system. Reduced sib-competition might help to explain the few cases where endosymbiosis has not resulted in the evolution of haplodiploidy, although sib-competition is hard to quantify (data might be available on factors that could correlate with the level of sib-competition e.g. the presence of gregarious clutches, the clutch size and how mothers protected their eggs). Such data might also in the future be able to distinguish between the importance of male killing vs. cytoplasmic incompatibility, as sib-competition is only a requirement for the former.

Recently the importance of genomic conflict in shaping defining characteristics of genomic organisation and key aspects of biology such as reproductive mode and genetic system has become apparent and received considerable attention (extensively reviewed in Burt & Trivers, 2006). However, few studies have actually made an attempt to test these hypotheses formally. Further comparative analyses such as the one undertaken here will help to increase our understanding of the evolutionary importance of genetic conflict.

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