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B. B. NORMARK

Genetic conflict and the dizygotic soma: on the adaptive significance of polar body transmission and the polyploid bacteriome in Pseudococcidae and Diaspididae

Abstract - Metazoans typically have a single-celled stage in the life cycle, a sexually produced zygote or apomictically produced asexual propagule. Some metazoans have vegetative reproduction, in which new individuals are formed from a large number of cells. Two families of scale insects, Pseudococcidae and Diaspididae, are apparently unique among Metazoa in that each individual is founded by two genomically distinct cells. One of these cells is a normal diploid zygote formed from fusion of ovum and sperm; it gives rise to the germline and most of the soma. The other cell is a polyploid secondary zygote, typically a pentaploid product of the fusion of the first (2N) and second (1N) polar bodies with a cleavage nucleus derived from the first zygote (2N). The secondary zygote gives rise to a specialised somatic tissue, the bacteriome, which houses vertically transmitted endosymbiotic bacteria. The bacteriome is analogous to endosperm in that it is a polyploid nutritive tissue with an enriched maternal genomic contribution, but differs in that it remains an integral and active part of the organism (at least in females) throughout ontogeny. Especially given the prevalence of brooding, and sex determination by paternal genome elimination in males, there is enormous scope for intra-genomic conflict in these insects, especially over the sex ratio. Consistent with the conflict hypothesis there is considerable variation in genomic composition of the bacteriome between species.

Key words: genomic imprinting, haplodiploidy, life cycle evolution, mycetocytes, sex-determination mechanisms.

INTRODUCTION

In the canonical life cycle of a metazoan, each multicellular individual develops from a single diploid cell, and thus has virtually the same genome (apart from a few somatic mutations) throughout its body. In some groups of metazoans there is also

facultative vegetative reproduction, in which adult organisms can fission and new individuals can develop from each many-celled fragment. But again, multicellular individuals wind up with virtually the same genome throughout. But a different mode of development is found in some groups of sternorhynchan insects. In these groups (some whiteflies and scale insects), individuals typically have a chimeric soma, with one organ – the bacteriome – having a different genomic constitution from the rest of the body. In the whitefly *Bemisia argentifolii*, bacteriocytes (mycetocytes) migrate from the mother's bacteriome (mycetome) into the developing oocytes (Costa et *al.*, 1996). Thus, the bacteriome in this species is directly derived from the maternal bacteriome, rather than from the zygote from which the other tissues develop. The relationship of the bacteriocytes' genome to the 'primary' genome in whiteflies is unknown. In the case of pseudococcid and diaspidid scale insects, the genome of the bacteriome is derived in part from the polar bodies produced during oogenesis (Tremblay & Caltagirone, 1973). Although a number of review papers have drawn attention to the tremendous diversity of scale insect genetic systems (Gullan & Kosztarab, 1997; Kosztarab, 1987; Nur, 1980; Nur, 1990), they have generally glossed over the profoundly strange fact of multiple, distinct nuclear genomes within individuals – probably because existing classifications of metazoan genetic systems do not even have categories for these phenomena. Here I draw attention to the uniqueness of the scale insect 'dizygotic soma,' place it in the context of genomic conflict, suggest some possible adaptive explanations, and suggest new research avenues and techniques.

FORMATION OF THE DIZYGOTIC SOMA

In Diaspididae (the armoured scales), a typical adult is a chimera of two genomically distinct cell lineages. Most of the insect's organ systems are diploid, derived in the normal way from development of a zygote formed from the fusion of an oocyte and a sperm nucleus. But its bacteriome, which houses intracellular endosymbionts on which it depends for nutrition, is pentaploid, and contains genetic material not present in the other tissues.

The polar bodies produced during oogenesis do not degenerate, but instead give rise to the bacteriome as follows (Brown, 1965): The first polar body (2n) fuses with the second polar body (1n) and one cleavage nucleus from the embryo (2n) to form a pentaploid (5n) cell, which undergoes many mitotic divisions to give rise to the bacteriome. Thus the bacteriome has two copies of the complete (2n) maternal genome (inbchcluding two copies of the half of the maternal genome that is not found in the rest of the soma) and one copy of the complete (1n) paternal genome – diaspidid males are all effectively haploid.

Individuals developing by this mode may be referred to as having a 'dizygotic soma.' I use the term 'dizygotic' to emphasize that the individual is founded by not one but two distinct and genetically different cells. One cell is a zygote in the normal sense of resulting from the fusion of a haploid oocyte with a haploid sperm. The other

(typically pentaploid) cell has a very different origin but is also a kind of zygote in the sense that it results from the fusion of cells and contains both maternal and paternal genomes. I use the term 'soma' to emphasize the exclusion of this second (pentaploid) cell lineage from the germline.

UNIQUENESS OF THE DIZYGOTIC SOMA

The closest parallel in nature to the dizygotic soma of scale insects is seen in the angiosperms. There, double fertilisation results in a triploid endosperm that is genomically distinct from the diploid embryo. The endosperm, like the bacteriome, derives its genome in part from a polar body, it has more maternal genome than paternal genome, and it is involved in the supply of nutrients. However, the endosperm is a temporary tissue associated with early development. Its genome is not incorporated into that of the plant. The scale insect bacteriome is very different in that it is an integral and active part of the organism throughout its life. A comparison with other developmental modes found in animals is given in Table 1.

AN ARENA OF GENETIC CONFLICT

We do not understand the significance of the unusual genomic constitution of scale insect bacteriomes. Though there are a number of published accounts of the cytogenetics of bacteriome formation (Brown, 1965; Hughes-Schrader, 1948; Nur,

Table 1 - A classification of developmental modes of Metazoa according to (a, rows) the number of cells from which an individual develops (rows) and (b, columns) whether individuals are genetically uniform (consisting of a clone differing only by somatic mutations or duplications) or genetically chimeric (consisting two or more genetically distinct clones of cells). This classification groups the development of diaspidids and pseudococcids with that of callitrichids (New World monkeys). Fetal callitrichid dizygotic twins share a single placental circulation system and are chimeric for blood cells throughout their lives (Haig 1999). This is a very limited chimerism compared to that seen in scale insects.

rdrw10	**genetically ~uniform**	**genetically chimeric**
rssingle cell	individuality: arthropods, vertebrates, etc.	multiple nuclei: *Icerya purchasi* (if outcrossed) (Royer, 1975) multiple mtDNAs: some bivalve molluscs (Zouros, 2000)
two cells	—	sib blood sharing: callitrichid primates (Haig, 1999) bacteriomes: diaspidids, pseudococcids
drw10 many cells	vegetative reproduction: cnidarians, anellids, etc.	interclonal fusion: cnidarians, bryozoans, &c. bacteriomes: whiteflies (Costa *et al.* 1996)

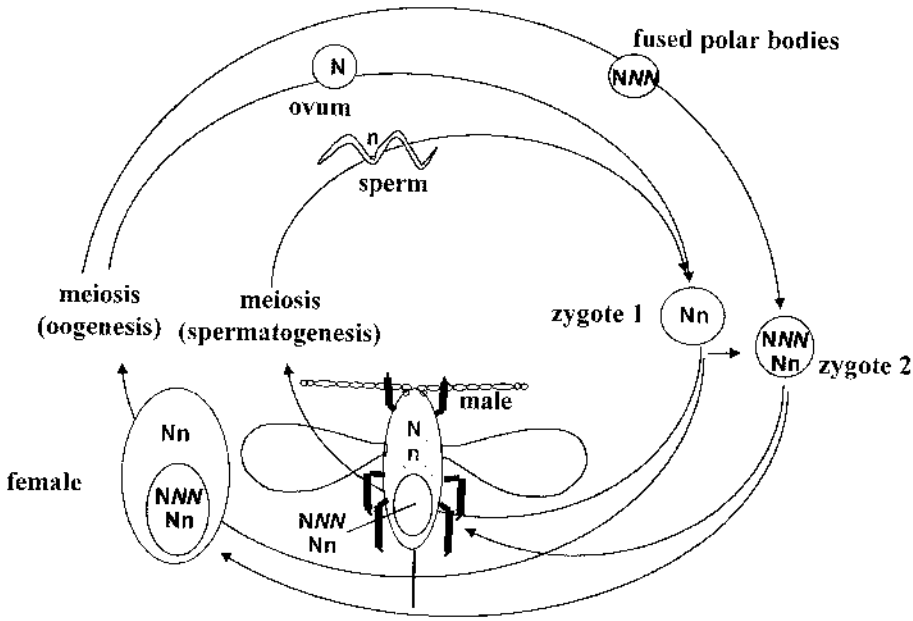

Fig. 1 - Schematic diagram of the genetic system of a diaspidid scale insect, illustrating the dizygotic soma. The haploid genome of the oocyte is indicated by a roman captial N. The other maternal haploid genome (the other half of the mother's diploid genome) is indicated by an italicized capital *N*. The paternal genome is indicated by a lower-case roman n. Males transmit only their maternal genomes; females recombine their maternal and paternal genomes during oogenesis and pass on both with equal likelihood. In this diagram, genomic imprinting is represented as occuring during gametogenesis: all genes are reset to 'maternal' (N or *N*) during oogenesis and 'paternal' (n) during spermatogenesis. The shaded areas represent the cell lineage containing cytoplasm and genetic material from the polar bodies and forming the bacteriome of the adult scale insect. The unshaded areas represent the cell lineage derived strictly from the oocyte, and forming the germ line and all the somatic tissues of the adult scale insect other than the bacteriome.

1977; Nur, 1980; Tremblay & Caltagirone, 1973), these are largely silent on the question of the adaptive significance of this novel feature of development. Nur (1977) suggests that the presence of additional genomes in the bacteriome may convey an adaptive advantage due to increased heterozygosity. To present-day evolutionary biologists, genetic chimerism immediately raises the spectre of conflict between the non-identical genomes, each of which is expected to favour itself or its kin over non-kin, but scale insect bacteriomes have apparently not been discussed in the scientific literature in this context, though other aspects of scale insect biology have been (Haig, 1993; 1997).

The following considerations may be relevant:

1) Bacteriome polyploidy has arisen in clades of scale insects that have germline paternal genome elimination. In this genetic system, which comprises the chromosome systems traditionally called 'lecanoid' and 'comstockiella' (Herrick & Seger, 1999), all individuals arise from diploid zygotes, but in some embryos the paternal set of chromosomes are heterochromatinized and silenced. The embryos with the silenced set of paternal chromosomes (functionally haploid) develop into males that transmit only their maternal set of chromosomes, whereas the embryos with active paternal and maternal chromosome sets (functionally diploid) develop into females. Thus, after a sperm nucleus has entered an oocyte, its genome may either be fully incorporated into the developing offspring, if that offspring is a daughter; or it may be inactivated and eventually eliminated, if that offspring is a son. This system leads to strong conflict between maternally and paternally inherited genes in a developing embryo over the sex of the offspring. Paternal genes only survive in daughters, so they are expected to favor 100% daughters; maternal genes survive in either sex and are expected to favor a sex ratio much closer to unity (Hamilton, 1967; 1979).

2) The bacteriomes house one or more lineages of intracellular bacteria that are strictly maternally inherited. Therefore, if the bacteria are able to influence the sex ratio, they should favor 100% daughters.

3) Adult female scale insects (especially in morphologically highly reduced groups such as Diaspididae) are extremely sedentary, and females remain in close association with their eggs until the eggs hatch and the crawlers disperse. Viviparity and other forms of brooding have evolved multiple times, further increasing the closeness and duration of contact between mother and offspring.

4) Although the paternal genome is inactivated in males in most of the soma, it remains active – it is not heterochromatinized – in the bacteriome. One can therefore easily determine the sex of a scale insect by cytogenetically examining any of its tissues – if the scale insect is a male, it will have only one euchromatic haploid complement of chromosomes – but this is not the case for the bacteriome, which is cytogenetically identical in the two sexes.

POLAR BODIES AND ENDOSYMBIONTS: ANTAGONISTS OR ALLIES?

The nature of the interactions between maternal genes present in the entire scale insect, maternal genes present only in the bacteriome, paternal genes, and endosymbionts, are quite unclear and potentially very complex. Here I will focus on interactions between the maternal nuclear genes in the polar bodies and cytoplasmically inherited elements such as the genomes of the endosymbionts.

Antagonism over sex determination. We are far from understanding the mechanisms of sex determination in scale insects, but it is generally thought that the mother is able to effectively control offspring sex ratio through the agency of cytoplasmic factors (Borgia, 1980; Haig, 1993), which in turn determine whether the paternal genome is eliminated. However, the paternal genome has sometimes (in groups of scale insects that apparently do *not* have bacteriome polyploidy) been able to 'break out' and regain

activity and transmissibility in males (Tremblay & Caltigirone, 1973; Herrick & Seger, 1999).

The a scale insect oocyte must admit into its cytoplasm two entities that have a very strong interest in influencing it to develop into a female: one is the bacterial flora it receives from its mother and the other is the sperm nucleus it receives from a male. Both of these will die without issue if the oocyte develops into a male. There will be very strong selection on sperm and on endosymbiotic bacteria favoring any variant that increases the probability of the oocyte developing into a female. The selection on the maternal genome to induce development as a male is necessarily weaker.

It is tempting to speculate that the mother sends in the polar bodies as 'reinforcements' against the subterfuges of these potential feminizing agents, in order to enforce a sex ratio close to the maternal optimum. However, this does not explain the lifelong activity of maternal genes in the polyploid sector, or its (usually) intimate association with endosymbiotic bacteria. Here is one possible adaptive scenario.

Prevention of son-killing through sexual crypsis. Assume that offspring are brooded, that the number of offspring is limited by the size of the brood chamber, and that the mother can continue to produce offspring until the brood chamber is full. Under these circumstances, offspring aborted very early in development (at a small size) can be replaced by later-produced offspring (Stearns, 1987). If these conditions apply in scale insects (as seems likely), they may select for suicidally altruistic endosymbionts. Consider a mutant endosymbiont that detected some cue indicating that it was residing in a male embryo and that responded to that cue by releasing a toxin that killed itself and the embryo. A number of bacteria are known to have a phenotype similar to this (Jiggins *et al.*, 2000). The brood chambers of females carrying such a mutant would tend to fill up with female offspring, and the suicidal male-killing endosymbiont lineage residing in them would have a reproductive advantage over non-son-killing endosymbionts (the strength of the advantage would be dependent on the efficiency with which dead offspring were replaced with live ones – no advantage if they were never replaced, up to a twofold advantage in an even-sex-ratio population in which they were replaced with perfect efficiency). One possible evolutionary response of the hosts to infection with such a bacterial lineage would be to deprive the bacteria of cues indicating maleness, and indeed to simulate femaleness in the tissue in which the endosymbionts reside – the bacteriome. Thus the activation of the paternal genome in the bacteriome of paternal-genome-eliminating scales may be an adaptation for gender crypsis, rendering male and female bacteriomes identical. However, the logic that applies to the endosymbionts applies equally to the paternal genome. Paternal genes, too, could benefit from suicide conditional on presence in a son. This may explain the quadruple dose of maternal genes in bacteriomes in which the paternal genes are active.

Cooperation favouring maternal kin. Although maternal genes and endosymbionts are expected to struggle over sex determination, in some other respects they have a striking concordance of interests. Natural selection is generally understood to be strongest at the level of the individual gene or the individual organism, and weaker at higher levels (such as selection between groups). One feature that endosymbionts and

polar bodies have in common is that this rule of thumb is not expected to be true for either of them. Endosymbionts are strictly clonal and maternally inherited. The genome of an endosymbiotic bacterium in one organism is virtually identical to the genome of the endosymbionts in that organism's siblings. Selection on endosymbionts is expected to favor traits that enhance the production of females by matrilines, even at the expense of individuals (as in the paradoxical selectively-favored suicide discussed above). Polar bodies are also extremely unusual in that they are expected to be *more* interested in the reproduction of maternal kin than they are in the reproduction of self. The second polar body (the haploid body that separates from the oocyte during meiosis II) contains all the maternal genes that are *not* in the oocyte and *none* of the maternal genes that *are* in the oocyte. Therefore, under outcrossing, it is non-kin to the oocyte (where the oocyte has a gene from the maternal grandmother, the 2nd polar body has a gene from the maternal grandfather, and vice versa). However, the 2nd polar body *is* related (as a full sib) to the mother's other offspring. Therefore, like the endosymbionts it is expected to favor the matriline at the expense of self. It is unclear how extreme matrilineal altruism might manifest itself in organisms that exhibit so little in the way of behavior. Advanced matrilineal sociality would seem to be precluded – at least in diaspidids – by females' the lack of legs beyond the first instar. However, intra-brood-chamber interactions between kin, and patterns of dispersal and settling, may turn out to be highly altruistic in these insects (Kasuya, 2000). The elimination of the paternal genome in males may turn out to be a matriline-favoring 'behavior' resulting from an interaction between endosymbionts (Hamilton, 1993) and maternally imprinted nuclear genes.

EVOLUTIONARY DYNAMICS OF THE POLYPLOID SECTOR

Arms races and conflicts are expected to drive rapid evolution. In many genera of diaspidids, the pentaploid bacteriome appears to be a fairly stable feature of development, suggesting that either genomic conflicts aren't really involved, or that it is a stable equilibrium outcome of ancient conflict. However, in other groups there is a great deal of variability in the genomic composition of the bacteriome, in the expression vs. suppression of the polar bodies' genomes, and in the association vs. dissociation of the polyploid sector with the endosymbiotic bacteria (Brown, 1965).

In pseudococcids the genomic composition of the bacteriome is highly variable. Frequently, pseudococcids have a monozygotic soma, with no participation of the polar bodies in bacteriome formation, and others have a pentaploid diaspidid-like bacteriome as described above (3n from polar bodies, 2n from cleavage nucleus of embryo). In others there may be a 2nd cleavage nucleus (7n = 3n polar bodies + 4n cleavage nuclei). Or the fused polar bodies (3n) may duplicate and fuse again, to yield hexaploid (6n) bacteriomes having no paternal genome, 2 complete copies of the mother's genome (4n) and 2 copies of the non-kin 2nd polar body (2n). But only a few species have

been investigated, and none, apparently, since the 1930's (Nur 1980, Tremblay & Caltagirone, 1973).

For diaspidids we have a more complete picture thanks to Brown's (1965) chromosome survey of 131 species. He found a few striking changes against the backdrop of a generally uniform pentaploid bacteriome. First, in a number of obligately parthenogenetic forms, the bacteriome is tetraploid. Second, there is sometimes an additional cleavage nucleus involved, as in some pseudococcids, resulting in a 7-ploid bacteriome. Most interestingly for the question of possible genetic conflict, there are some cases (a) in which the pentaploid sector is formed but does not serve as the bacteriome, the endosymbionts residing elsewhere (*Lindingaspis*) and (b) in which heterocrhomatisation of 3 haploid genomes is seen in bacteriomes from both sexes, which Brown interprets as inactivation of the polar body genomes (*Chionaspis, Aulacaspis, Pinnaspis*). A similar heterochromatisation of polar body genomes has recently been observed in the pentaploid endosperm of a monocot (Buzek *et al.*, 1998).

PROSPECTS

Very little research has been done on the dizygotic soma of scale insects since the work of Brown (1965) and Nur (1977) decades ago. Ongoing work on the diversity, physiology, and genomics of bacterial endosymbionts of scales by T. Fukatsu (pers. comm.) and P. Baumann (pers. comm.) should help to clarify the function of the bacteriome. To understand the role of maternal vs. paternal genomes, the genetic basis of sex determination and the possibility of genomic imprinting in females needs to be investigated – including the possibility of differential expression of polar-body-derived vs. oocyte-derived alleles.

The diversity of genomic compositions of bacteriomes across species, as well as evolutionary novelties such as heterochromatisation of the polar body genomes in some lineages and dissociation of the bacteria from the bacteriome in others, make one hopeful that phylogenetic and comparative studies may shed light on the evolution of these interactions. Use of flow-cytometric methods should greatly simplify the search for genomic variants (with different ploidy levels) within and between species (Normark, 1996), and we can even hope that intraspecific variants may be found that are amenable to classical genetic studies.

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BENJAMIN B. NORMARK - Department of Entomology, Fernald Hall, University of Massachusetts, Amherst, Massachusetts 01003, USA. E-mail: bnormark@oeb.harvard.edu.