Genomic Imprinting

Rolf Ohlsson (Ed.)

An Interdisciplinary Approach

Imprinting and Paternal Genome Elimination in Insects

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In R. Ohlsson (ed), *Genomic Imprinting: An Interdisciplinary Approach*, pp 41-71. Springer, Berlin (1999).

1 Introduction

In many insects and other arthropods, males transmit only maternally inherited chromosomes (White 1973; Brown and Chandra 1977; Nur 1980, 1990a,b,c; Bell 1982; Bull 1983; Lyon and Rastan 1984; Lyon 1993; Wrensch and Ebbert 1993; Brun et al. 1995; Borsa and Kjellberg 1996). This remarkable genetic asymmetry can result from any of three principal systems of paternal genome exclusion, each of which has evolved several times. The most familiar and widespread exclusion system is *arrhenotoky*, in which fatherless males develop from unfertilized eggs and therefore lack paternal chromosomes at all stages of development. Most arrhenotokous systems are genetically haplodiploid, but a few are based on other modes of inheritance (see Nur 1980, 1990c; Bell 1982; Suomalainen et al. 1987). In the two other kinds of exclusion systems, a male's paternally inherited chromosomes are actively *eliminated*: males begin life as seemingly conventional diploid zygotes but then either (1) lose their paternal chromosomes during embryonic development, becoming true maternal haploids (*embryonic* elimination), or (2) exhibit dramatically non-Mendelian patterns of meiosis and spermiogenesis, such that mature sperm carry only maternal chromosomes (*germline* elimination). To denote their formal (transmission-genetic) similarity to haplodiploid arrhenotoky, the embryonic and germline elimination systems are often characterized as *parahaplodiploid* or *pseudoarrhenotokous*.

These three routes to exclusive transmission of maternally inherited chromosomes are connected both mechanistically and historically. For example, genomic imprints direct both embryonic and germline elimination in many cases, and embryonic systems have evolved from germline systems in some cases (Nur 1980). Arrhenotokous systems do not appear to depend on imprints, but arrhenotoky may have evolved (at least in a few cases) from embryonic elimination, by deletion of the vestigial requirement for fertilization to initiate male development (Bull 1983; Haig 1993a; Sabelis and

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Nagelkerke 1993; Brun et al. 1995; Borsa and Kjellberg 1996). The systems are related dynamically, as well, in that germline elimination, embryonic elimination, and arrhenotoky all seem to represent evolutionary responses to underlying intragenomic "conflicts of interest" between a male's maternally and paternally inherited chromosomes (Brown 1963, 1964; Bull 1983; Haig 1993a,b).

Here we briefly survey paternal genome elimination (PGE) in insects. We emphasize two taxa in which the phenomenology of PGE, and the role of imprinting, are especially well understood. We begin with the dung gnat *Sciara coprophila* (whose study first gave rise to the concept of genomic imprinting), and then turn to the scale insects (a group with thousands of species exhibiting almost every known variation on the theme). Several mechanistic features of scale-insect PGE systems can be interpreted as adaptive evolutionary responses, by maternally inherited genes, to paternal counter adaptations for preventing elimination. We develop a model in which alternating evolutionary "moves" and "countermoves" (fixations of mutations advancing maternal or paternal interests) give rise to the observed diversification of scale-insect PGE systems. The model generates testable predictions about the mechanics and the phylogenetic histories of these systems.

Recent discussions of mammalian imprinting have tended to blur a critical distinction between imprints and their effects. Reference to an "imprinted gene" has come to connote both the *existence of the heritable mark* (the imprint) and the resulting *differential behavior* (the action cued by the imprint). Students of chromosome elimination have usually been careful to observe this distinction (e.g., Nur 1990b), perhaps because its significance is easier to appreciate in these systems than in mammalian systems, where the cued effect is differential gene expression. We argue that mammalian imprinting, too, will be better understood when the imprint itself is clearly distinguished from the actions that may or may not occur in response to it.

Some elimination systems appear not to depend on imprints. For example, intracellular parasitic bacteria of the genus *Wolbachia* manipulate host chromosomes in many arthropod taxa, causing hybrid incompatibility, female parthenogenesis (thelytoky), feminization of otherwise male-destined embryos, and paternal genome elimination (see Breeuwer and Werren 1990; O'Neill et al. 1992, 1997; Rousset et al. 1992; Moran and Baumann 1994; Werren et al. 1995; Lassy and Karr 1996; Schilthuizen and Stouthamer 1997; Werren 1997). *Wolbachia* are sometimes said to imprint host chromosomes destined for destruction, but there is no need to invoke a heritable mark that persists for more than one mitosis in the *Wolbachia*-mediated systems that have been studied cytogenetically. Similarly, the ultraselfish supernumerary *PSR* chromosome of the wasp *Nasonia vitripennis* causes efficient paternal genome elimination (Nur et al. 1988; Werren 1991), but this, too, can be explained by

direct effects of *PSR* on the chromosomes that are about to be destroyed. These parasite-mediated chromosome eliminations have much in common with imprint-cued eliminations, but close attention to some important differences (discussed below) should lead to better understanding of the mechanisms and the evolutionary origins of these phenomena. One interesting possibility is that arthropod species infected by *Wolbachia* might become relatively more likely to evolve imprint-mediated elimination systems, if such infections tend to promote the establishment of predisposing conditions that would otherwise be less common (see Varmuza and Mann 1994b).

2 Paternal Chromosome Elimination in *Sciara*

More than 70 years ago, Metz (1925) described meiosis in the male of *Sciara coprophila* (reviewed by Du Bois 1933; Metz 1938; White 1973; Brown and Chandra 1977; Lyon and Rastan 1984; Gerbi 1986). He found that a complete haploid chromosome set is eliminated in meiosis I (Fig. 1d), pinched off from the spermatocyte in a bud (see Fuge 1997). As in many insects, there is no recombination in males. The eliminated chromosomes are paternal.

In addition to this dramatic and complete paternal genome elimination, the chromosome cycle includes several other unusual imprint-cued behaviors, as illustrated in Fig. 1. Male and female zygotes receive one X chromosome and a haploid set of autosomes from the female pronucleus (X^mA^m), but they receive two Xs and an autosomal set from the sperm ($X^PX^PA^P$). During the seventh embryonic round of cleavage divisions (Fig. 1b) the *somatic* precursor nuclei lose two X^Ps (in males) or one X^P (in females) by "anaphase lag": the X^P chromatids fail to disjoin and are left behind on the metaphase plate (Du Bois 1933; Fuge 1994, 1997; de Saint Phalle and Sullivan 1996). As a consequence, males become somatically X^mO and females become X^mX^P. Later, *germline* cells enter an unusual, extended interphase in which the chromosomes are partly condensed and visible ("prochromosomes"); one set is much less condensed than the other, in response to a differential imprint (Fig. 1c; Berry 1941; Rieffel and Crouse 1966). In both males and females one X^P is "extruded" or "dragged" from this interphase nucleus into the cytoplasm, where it later disappears. Thus, the male and female germ lines both become fully diploid ($X^mX^PA^mA^P$).

During meiosis I in the male, the paternal set segregates away from the maternal set and into a cytoplasmic bud that is eliminated from the spermatocyte (Fig. 1d), as first observed by Metz. During meiosis II, the remaining maternal *autosomal* chromatids disjoin and one set is eliminated in a bud, but the two X^m chromatids fail to disjoin and migrate precociously to the sperm-destined pole. The surviving chromosomes ($X^mX^mA^m$) are packaged into a single sperm (Metz 1938). Thus, the male provides double-X sperm to

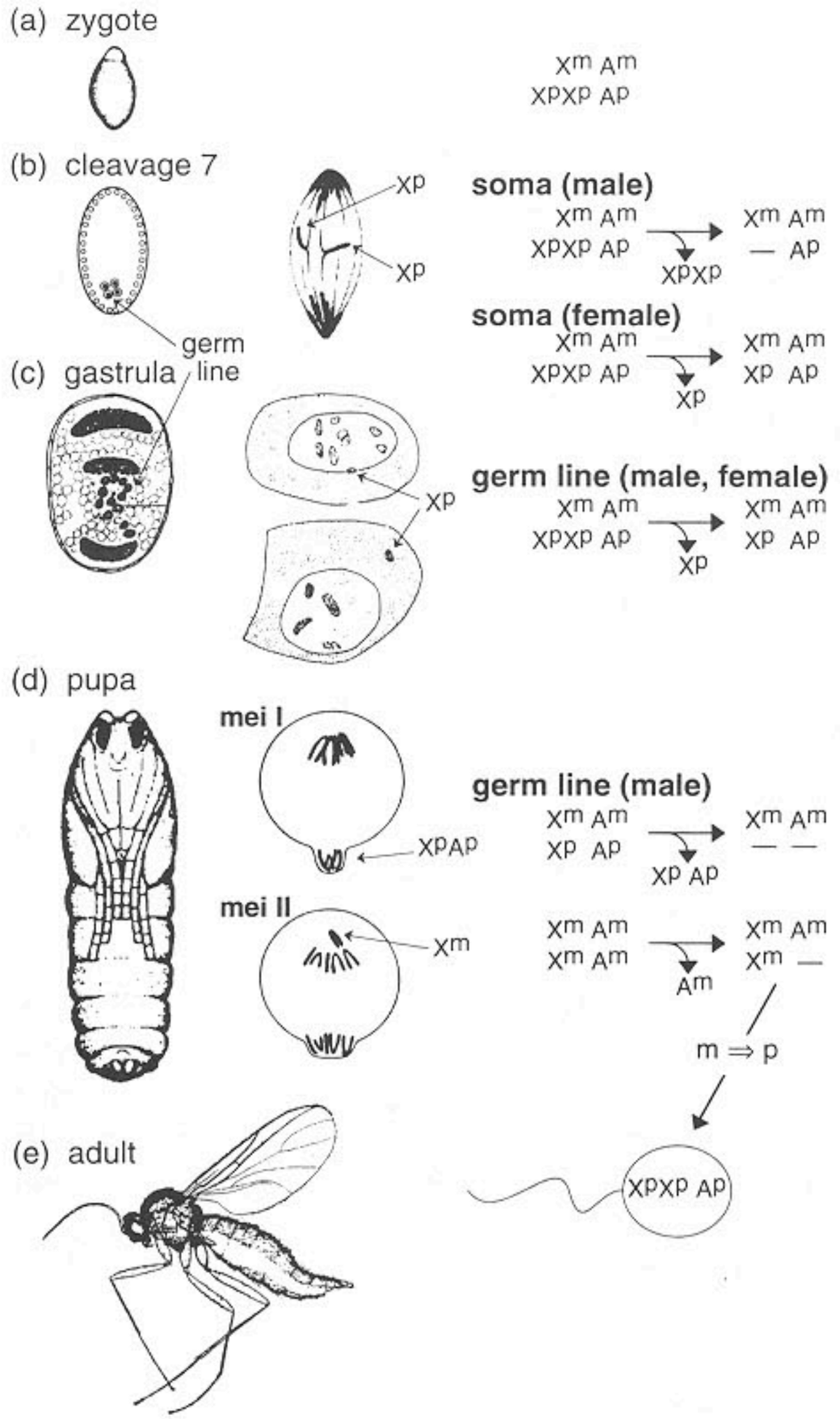


Fig. 1a–e. *Sciara* chromosome cycle. Developmental stages are shown at the *left*; corresponding cytogenetic events are shown in the *middle* and then represented diagrammatically at the *right*. See text for a more complete account. Authoritative reviews are provided by Metz (1938) and Gerbi (1986); accessible overviews are provided by White (1973) and by Lyon and Rastan (1984). Here, for simplicity, we ignore the germline limited (L) chromosomes found in most sciarids. ***a*** The zygotic genotype is diploid, but it includes two paternal X chromosomes (X^PX^P). ***b*** In the seventh cleavage, one cleavage after nuclei migrate to the surface of the embryo and germline cells are set aside internally, all somatic precursors lose two X^Ps (in males) or one X^P (in females), during mitosis, by anaphase lag (mitotic figure from Du Bois 1933). ***c*** In the late gastrula, one X^P is eliminated from each interphase germline cell (drawings from Berry 1941). ***d*** Meiosis occurs in the pupa. These drawings from Du Bois (1933) show elimination of the paternal set in meiosis I, elimination of a maternal chromatid set in meiosis II, and precocious nondisjunction of X^m chromatids in meiosis II. The surviving maternal chromosomes are packaged into a sperm and will be inherited as paternal. ***e*** The adult male provides double-X^P sperm to fertilize eggs, completing the cycle. (Insect drawings *a*, *d*, *e* from Cole 1969)

fertilize all eggs, completing the cycle. The imprints have been reversed. The male's *maternal* autosomes and X chromosome will behave as *paternal* in his offspring.

2.1 Imprints Defined

In 1938 Metz wrote: ". . . the *difference in behavior* [of chromosomes] is due to a . . . *difference* between [them that] . . . *is impressed* on [them] in the *preceding generation* by the sex of the parent. . . . This . . . *modification persists* for only one generation and is *reversible*." This statement summarizes several original and important insights:

1. Differential chromosome behavior indicates the existence of differential modifications that cue the behavior. Metz used the word "*impressed*." Crouse (1960) later borrowed the term "imprint" from ethology, establishing the modern usage.
2. It is not clear whether the imprints mark the maternal or paternal chromosomes, or both (Lyon and Rastan 1984). Do "paternal imprints" cue elimination, or do "maternal imprints" confer protection? Ultimately, an imprint is a "*difference*" between the maternal and paternal homologs, not an absolute or intrinsic state of either one.
3. The imprints "*persist*" from the parent, through the zygote, at least to spermatogenesis at the end of the male germ line. Thus, the imprints must be replicated along with the chromosome's genetic material for many cell generations, as epigenetic marks.
4. The imprints are "*reversible*". A male transmits his maternal genes as paternal, and a female transmits her paternal genes as maternal.

2.2 When Are Imprints Laid Down?

The chromosome set in the male's sperm has just behaved as a maternal set in meiosis I, but it will behave as a paternal set in the progeny. Thus the imprints can reverse no earlier than meiosis II, or more plausibly, during subsequent spermiogenesis. Alternatively, a paternal identity could be established much later, in the egg, after fertilization but before the first zygotic mitosis (see Latham et al. 1995). In many organisms, the two pronuclei proceed in close apposition, but not fused, through an interphase to the first mitosis, in which the two pronuclear chromosome sets finally are brought together on the metaphase plate. Even at this point, and through several subsequent mitoses, the sets may continue to occupy distinct volumes in the diploid nuclei (*gonomery*: reviewed generally by Wilson 1928; in various insects by Huettner 1924 and Zissler 1992; in humans by Sathananthan 1997;

in interspecific plant hybrids by Leitch et al. 1991; and in fish by Wilson 1928). Diploid males occasionally are produced by parthenogenesis in the scale insect *Pulvinaria hydrangeae* (Coccidae). Both chromosome sets are maternal, yet at the appropriate time in embryogenesis (see below), one set appears to acquire all the properties of a paternally imprinted set (Nur 1963). This suggests that at least in coccids, the imprints may be laid down during early embryogenesis (Chandra and Brown 1975; Brown and Chandra 1977; Nur 1980, 1990b).

Early embryonic imprinting could be common. In the mouse, the two pronuclei also remain separate until the first mitosis; the male pronucleus is more swollen than the female pronucleus (Donahue 1972a,b; Wright and Longo 1988) and may begin S phase earlier (Abramczuk and Sawicki 1975; but see Howlett and Bolton 1985). Pronuclear swapping experiments show that some mouse imprints are laid down before the first mitosis (reviewed by Surani 1986), but additional imprints could be laid down afterward, since the two chromosome sets lie in different parts of the nucleus for at least two more cleavages (Odartchenko and Keneklis 1973; Brandriff et al. 1991). In short, there is reason to suspect that even "paternal imprints" may often be of *maternal* origin, in the sense that they are established in the early embryo by machinery that was present in the egg before fertilization.

2.3 *XCE* and Action at a Distance

Crouse (1960, 1979) identified a locus on the *Sciara* X chromosome that controls the various differential X behaviors (anaphase lag, interphase extrusion, and meiosis II nondisjunction). When this locus is translocated to an autosome, the autosome exhibits "a program of behavior . . . [that is] indistinguishable from the one enacted normally by the X" (Crouse 1979). This "X Controlling Element" (*XCE*) lies between the centromere and the proximal telomere on the acrocentric *Sciara* X, but when translocated onto an autosome, at a location well away from the centromere, it directs actions in *cis* at great distances. An imprint is clearly involved (Crouse 1979), but where is it? If the imprint marks the centromere, then *XCE* acts on that information over a large distance; or alternatively, if the imprint occurs at *XCE*, then the imprint cues action at a large distance (i.e., at the centromere). Thus an imprint and the locus affected by it may lie far apart.

As Du Bois (1933) observed (Fig. 1b), during the seventh somatic mitosis the X^p centromeres appear to pull apart normally, but their progress is arrested by the chromatids, which remain firmly joined together, causing anaphase lag and elimination of the chromosome. Recent studies confirm this basic observation, add spectacular detail, and suggest that anaphase lag is caused primarily by an imprint-cued failure of chromatid separation rather than by centromere dysfunction (de Saint Phalle and Sullivan 1996). This interpretation implies that *XCE* (and/or the imprint) may affect different cellular structures at different times and places. Although anaphase lag may be caused by a failure of sister-chromatid separation, neither interphase extrusion of an X^p from the germ line, nor meiotic elimination in males, seems likely to be caused (at least primarily) by this mechanism.

The better known but more recently discovered *Xce* locus of the mammalian X chromosome controls the inactivation of its X. Like the *XCE* of *Sciara*, this *Xce* acts in *cis* over great distances (reviewed by Lee and Jaenisch 1997; Heard et al. 1997). The *Xce* region includes the *Xist* gene, which must be expressed to inactivate the X that carries it. *Xce* bears an imprint that influences the expression of its associated *Xist* in extraembryonic tissues, such that the paternal X is preferentially inactivated. In some marsupials, one X (probably paternal) is *eliminated* from the female's soma (reviewed by White 1973; Brown and Chandra 1977; Hayman and Rofe 1977).

Sciara males express both maternal and paternal genes in most tissues, but apparently not in all. Crouse (1966; reviewed by Brown and Chandra 1977) crossed parents carrying balanced X-autosome translocations, and recovered exceptional males with no X^m ($A^mA^pX^pX^p$). These males eliminated one X^p in the germ line, becoming X^pO. They were sterile, although males with just one maternal X in the germ line (X^mO) were fertile. Crouse concluded tentatively that while a single X^m can support germline function, a single X^p cannot. This implies that the imprint(s) also affect expression of genes needed for germline function.

3 Imprints and Their Effects

A single persistent mark on the *Sciara* X chromosome could cue its various imprint-directed behaviors at different times and places in development, even though the mark caused no visible action in most cells at most times. Similarly, imprints on *Sciara* autosomes appear to cue action only in the spermatocyte; in particular, they do not appear to affect somatic gene expression (Smith-Stocking 1936), as they do in many systems. In any system, we can be aware only of imprints that cue actions we observe. Thus, imprints could exist that cue subtle effects that no one has noticed, and many imprints could have no effects.

If imprints are viewed as distinct from the mechanisms that read them, then it is easier to imagine action at a distance, or several different actions cued by one imprint. For example, methylation of a CpG dinucleotide could affect the functioning of the element within which it sits (e.g., a promoter) in very different ways, depending on aspects of its nuclear environment (e.g., populations of transcription factors and other DNA-binding proteins).

Various mechanisms of imprint-cued action toward specific loci, domains, or chromosomes could evolve independently, with different effects (e.g., elimination or inactivation), and in response to different selective forces.

One important set of selective forces derives from conflicts of interest among genes within a genome (see Haig and Trivers 1995). For example, the maternally and paternally inherited alleles at a locus metaphorically compete for transmission into viable gametes. A statistical bias (meiotic drive), too small to be detected in experiments of practical size, could generate a decisive fitness advantage for the favored allele. The elimination of paternally inherited chromosomes in male *Sciara* dramatically boosts the transmission of maternally inherited genes (see Brown 1963, 1964; Hartl and Brown 1970; Bull 1979, 1983; Haig 1993a,b).

When rare, a maternally inherited allele that caused males to eliminate their paternal chromosomes would enjoy a twofold reproductive advantage because it would appear in all sperms (rather than in half of them). Similarly, a rare allele expressed in mothers that caused their sons to eliminate the father's chromosomes would appear in half (not one quarter) of their sons' sperms. As such an allele increases in frequency, the size of its advantage decreases because it more often causes its own elimination in males, but selection continues to favor it right up to fixation (Brown 1964; Hartl and Brown 1970; Bull 1983). In principle, such a strategy should be feasible in many species with parental imprints, and, in fact, PGE has evolved independently in diverse taxa (White 1973; Brown and Chandra 1977; Nur 1980, 1990a; Bell 1982; Bull 1983; Lyon and Rastan 1984; Nur et al. 1988; Stuart and Hatchett 1988; various chapters in Wrensch and Ebbert 1993; Brun et al. 1995). For example, paternal chromosomes are not transmitted by males of the Hessian fly, *Mayetiola destructor* (Cecidomyiidae), and maternal chromosomes stain especially heavily ("positive heteropycnosis"; White 1973) in both the male and female germ lines, visibly revealing a differential imprint (see White 1973; Brown and Chandra 1977; Stuart and Hatchett 1988; Formusoh et al. 1996). Following a discussion of PGE in scale insects, we briefly describe some less well known but seemingly similar systems in bark beetles and mites.

PGE also occurs in several all-female "hemiclonal" fishes and amphibians with "hybridogenic" reproduction (see Schultz 1977, 1989; Dawley and Bogart 1989; Carmona et al. 1997), and in the more recently characterized hybridogenic stick insects of the *Bacillus rossius-grandii* complex (Mantovani and Scali 1992; Tinti and Scali 1992, 1993, 1995). There may even be hybridogenic plants (Davies 1974; Heslop-Harrison 1990). The hybridogenic fish *Poeciliopsis monacha-lucida* has been especially well studied (see Schultz 1989). Females mate with males of a closely related bisexual species, *P. lucida*. The resulting offspring are all female; these "sperm parasites" (Carmona et al. 1997) express their paternally inherited genes but eliminate the paternal genome in meiosis I of *oogenesis* (Cimino 1972). Thus their eggs carry a clonally propagated haploid maternal genome. By eliminating (and reacquiring) the entire paternal genome each generation, hybridogens maintain populations of diverse F_1 genotypes (see Schultz 1969; White 1978). Like other unisexual vertebrates, hybridogens typically arise as interspecific hybrids, and some hybridogenic "species" have multiple origins (see Dawley and Bogart 1989; Quattro et al. 1991).

Given the very strong selection in favor of PGE, why is it not even more common than it is? It evolves under many different circumstances, and it can remain viable for tens or hundreds of millions of years (unlike fully asexual reproduction; see Bell 1982). Some necessary or predisposing conditions (Whiting 1945; Brown 1977) must be relatively uncommon, but what are they? In our view, the relative rarity of PGE is an interesting and important problem that deserves more attention than it has received.

4
Paternal Chromosome Elimination in Scale Insects

A great diversity of germline and embryonic elimination systems has evolved in the "coccids" (*sensu lato*, now Coccoidea, hence *coccoids*), a suborder within Homoptera that includes mealybugs and other scale insects (Hughes-Schrader 1948; Brown 1977; Brown and Chandra 1977; Nur 1980, 1990a). The principal PGE systems are named after the taxa in which they were first described. The relatively simple *lecanoid* germline system is ancestral to the more complex *Comstockiella* germline system, which in turn gave rise (apparently several times; see below) to *diaspidid* embryonic elimination. PGE may also occur in the testicular tissues of hermaphroditic *Icerya* (Margarodidae) (reviewed by Brown and Chandra 1977; Nur 1980).

4.1
Events in the Early Embryo

In male embryos of species with germline elimination (lecanoid and Comstockiella systems), all chromatids disjoin normally at the sixth mitosis (Nur 1967), but in the next interphase the entire paternal chromosome set ("P-set") fails to decondense; the paternal chromosomes remain transcriptionally inactive, and they stain darkly. This heterochromatic state replicates faithfully in the germ line and in most somatic cell lineages (Fig. 2).

In male embryos of species with embryonic (diaspidid) elimination, paternal chromatids fail to disjoin in an early cleavage division, shortly before the stage at which they would become heterochromatic in species with lecanoid or Comstockiella systems (Brown 1965; Brown and Chandra 1977; Nur 1980). These paternal chromosomes are eliminated by anaphase lag from the nuclei that form on completion of this mitosis, leaving the embryo in a truly haploid state. If a P chromosome escapes elimination at this mitosis, then it remains condensed until the next mitosis, when it is eliminated (Brown 1965). This

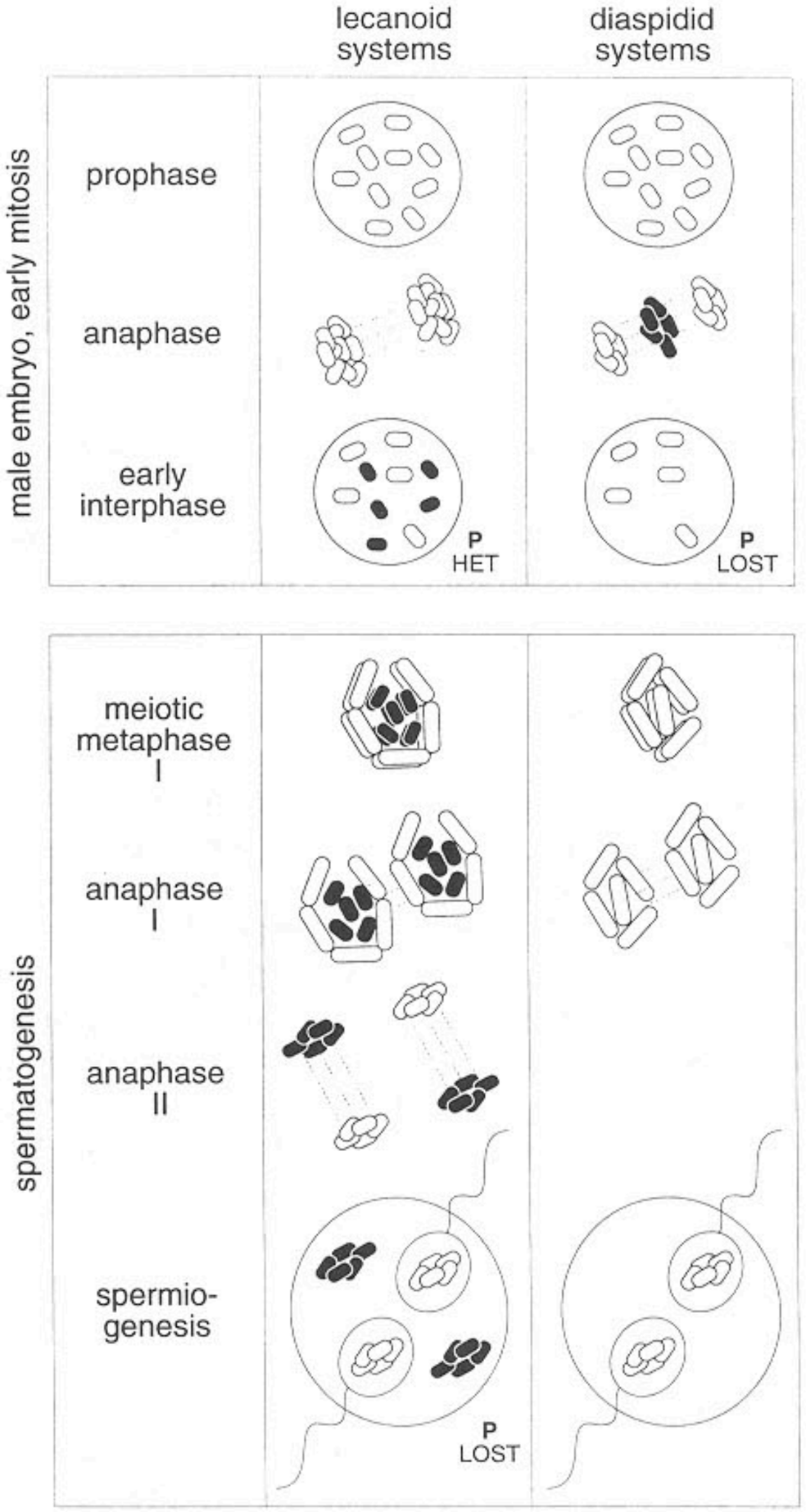


Fig. 2. Paternal genome elimination in male coccoids. Chromosome behaviors in the male embryo and in spermatogenesis are contrasted in species with lecanoid (*left*) and diaspidid (*right*) chromosome systems (reviewed by Brown and Chandra 1977; Nur 1980). Paternal chromosomes are shown as filled (*black*) at stages where they stain or behave differently from maternal chromosomes. *Top panel.* Events at an embryonic mitosis: prophase, anaphase, and the following early interphase. On entering the next interphase, lecanoid paternal chromosomes fail to decondense (heterochromatin). In diaspidid males, the paternal chromosomes are eliminated by anaphase lag. *Bottom panel.* Spermatogenesis. Meiosis in coccoids is inverse, such that the first segregation is equational (sister chromatids disjoin), while the second segregation is reductional (maternal and paternal homologs disjoin). Meiosis I in lecanoid males gives rise to four distinct sets of chromatids: a P-set and an M-set at each pole (first and second images); recombination being absent, meiosis II separates the four sets (third image); in spermiogenesis only the M-sets are packaged into sperm, while the P-sets degenerate (fourth image). In diaspidid spermatogenesis the P-set is absent; meiosis II does not occur; in spermiogenesis the two sets of maternal chromatids are packaged into sperms

suggests that heterochromatization is an essential early step in diaspidid elimination, as it also appears to be in the lecanoid and Comstockiella systems (see below).

Brown and Nelson-Rees (1961) proved that the heterochromatic chromosomes of lecanoid males are paternal by subjecting fathers to high doses of X-irradiation and then observing that their sons carried fragmented heterochromatic chromosomes. Even small fragments can persist and segregate mitotically, because coccoid chromosomes are holocentric (i.e., centromeric function is distributed along the chromosome) (Hughes-Schrader and Ris 1941). Two other conclusions emerged from these experiments. First, the imprint (like centromere function) is *distributed widely* along the length of each chromosome, because fragments behave as paternal in males (Brown and Nelson-Rees 1961; Nur 1990b). Second, the heterochromatic paternal set appears to be *genetically silent*, because sons do not express X-ray-induced dominant lethal mutations when the irradiated chromosomes are inherited from their fathers (in fact, the sons show nearly normal viability), whereas daughters do express such mutations (resulting in poor viability). This inference was corroborated by subsequent studies of nonlethal mutations with visible phenotypes, in which sons expressed the mutations only when inherited from their mothers (Brown and Wiegmann 1969; Nur 1990b).

4.2 Spermatogenesis

As in all Homoptera, meiosis in coccoids is "inverse" (reviewed by Hughes-Schrader 1948; White 1973). In the absence of recombination (e.g., in the males of species with lecanoid or Comstockiella PGE), chromatid-chromatid (equational) disjunction in meiosis I precedes maternal-paternal homologue (reductional) disjunction in meiosis II. The paternal chromosome set enters spermatogenesis in the heterochromatic state that was established at the sixth embryonic mitosis (Fig. 2). At metaphase I the more highly condensed paternal set is "clumped" together at the center of the metaphase plate, nearly surrounded by the maternal set (Schrader 1923; Nur and Brett 1988). Thus the maternal and paternal sets occupy distinct locations at the onset of meiosis. After chromatids disjoin, the spatial arrangement transforms, such that by metaphase II the two sets lie on separate plates. This polarity appears to be the immediate cause of the dramatically non-Mendelian segregation that occurs during anaphase II, when the entire maternal and paternal sets segregate to opposite poles. The heterochromatic P-set does not give rise to sperm (at least not usually, as discussed further below).

The Comstockiella system is a variant of the lecanoid system in which some or all of the paternal chromosomes are eliminated precociously, just prior to meiotic prophase, by an unknown mechanism (they simply disappear!), with the remainder being eliminated later, in meiosis II, by the usual lecanoid

mechanism (Kitchin 1970, 1975). This system appears to represent an important transition toward diaspidid-style complete early elimination (Nur 1980). In diaspidid spermatogenesis (Fig. 2), meiosis II is bypassed and the two single-chromatid maternal chromosome sets that segregate in meiosis I are packaged into two sperm.

4.3 Are Gametic or Early Embryonic Imprints Later Converted to Heterochromatin?

Heterochromatin exhibits replicative persistence and reversibility, so the original imprints could be converted to differential chromatin states and then lost in early embryogenesis. More generally, a variety of different kinds of imprints could exist at different loci, in different developmental contexts, and in different taxa.

Studies of supernumerary (B) chromosomes in lecanoid males (Nur 1962; Nur and Brett 1988) suggest that the paternal set is eliminated because it is heterochromatic. Both paternally and maternally inherited B chromosomes exit premeiotic interphase already condensed, but in prophase they take on the less condensed appearance of the maternal A chromosomes, and they remain outside the clump of paternal A chromosomes in metaphase I. In meiosis II they tend to segregate preferentially with the maternal chromosomes, and are thereby included in sperm (Nur 1962; Nur and Brett 1988). This "selfish" accumulation mechanism maintains them within populations, despite their mildly deleterious effects on the individuals that carry them. Certain genetic backgrounds suppress this driving behavior of the B chromosomes, apparently by preventing them from converting to the euchromatic state of the maternal set in prophase I. These B chromosomes (still fully condensed) fail to escape the central paternal chromosome clump at metaphase I and thereby suffer elimination (Nur and Brett 1988). Thus, being heterochromatic would appear to be necessary, and perhaps sufficient, to ensure subsequent elimination in lecanoid systems, and, as discussed above, heterochromatization also appears to play a mechanistic role in diaspidid elimination (see Fig. 2).

4.4 Conditions for the Evolution of PGE in Coccoidea

All coccoids with PGE lack sex chromosomes and have achiasmate male meiosis, but the basal (non-PGE) coccoid lineages retain conventional XX-XO sex determination and male recombination (Fig. 3). Thus, heterogametic sex determination must have been converted to environmental (maternal) control in the line that gave rise to the ancestral PGE system. Conventional male-heterogametic sex determination is incompatible with PGE, because a male who transmitted his maternal chromosomes would thereby transmit an X in

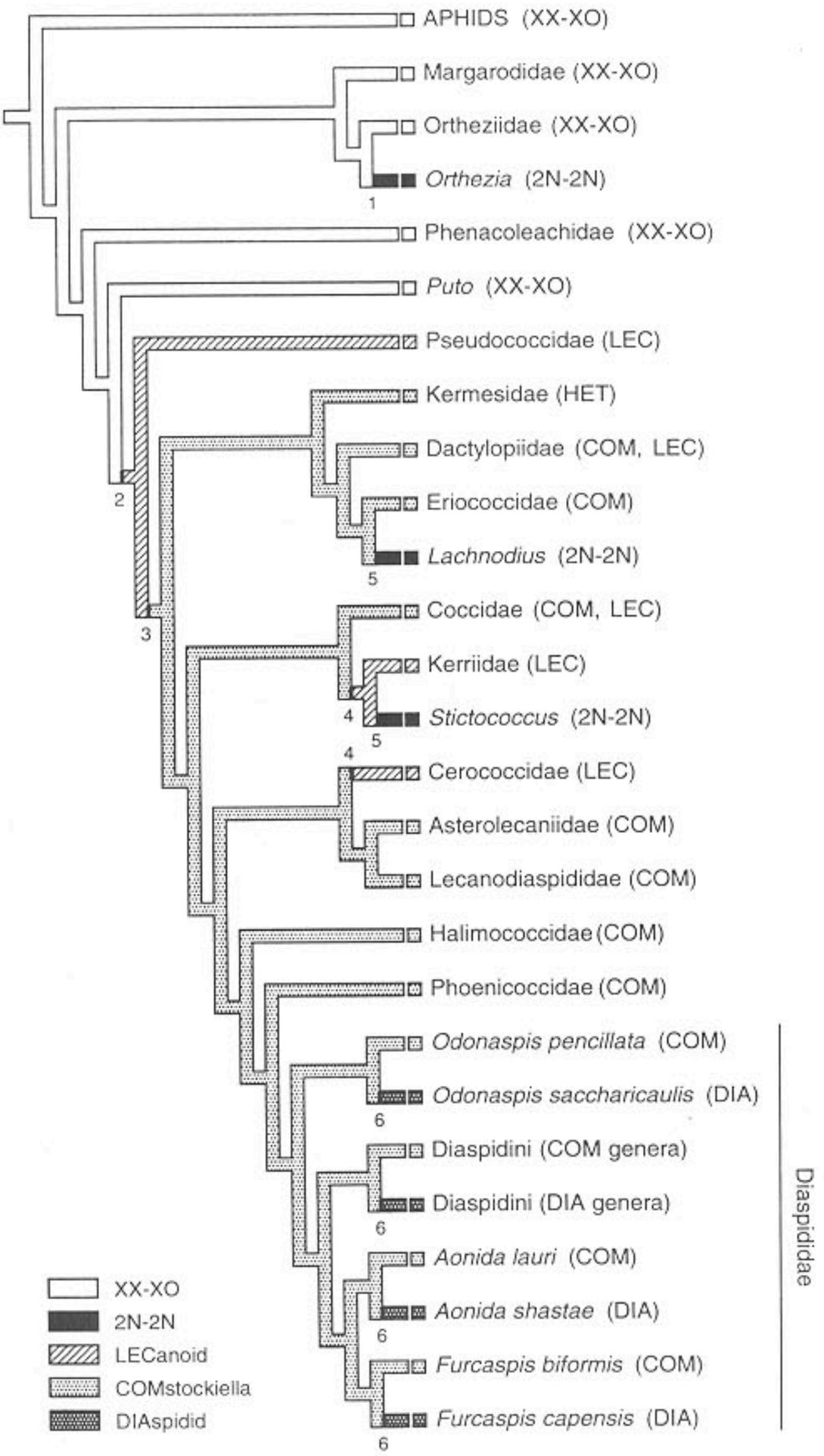
every sperm. If every egg also carried an X (from the XX mother), then every offspring of a male exhibiting PGE would be an XX genetic female (see Bull 1979). *Sciara* seems to violate this principle since it has XO males, but offspring sex is determined by the *mother*, not the *father's gamete*. The male germ line is fully diploid ($X^mX^pA^mA^p$), and all sperm carry $X^mX^mA^m$ chromosome complements. The male *soma* becomes *pseudo*-heterogametic ($X^mOA^mA^p$) during embryonic *development*, through differential chromosome *elimination*, as described above (Fig. 1). Thus the "sex chromosomes" of *Sciara* retain only vestiges of a conventional role in gametic sex determination. An analogous transformation of heterogamety occurs in aphids, where parthenogenic females (XX) produce sexual females (XX) and males (XO); in a male-destined egg, one X is eliminated during oogenesis. Males then produce 100% X-bearing sperm (and XX daughters) by aborting spermiogenesis of "O-bearing" meiotic products (reviewed by Blackman 1987).

Why might a system of heterogametic sex determination change to one of maternal control? Haig (1993a,b) points out that a meiotically driving X chromosome would create a female sex-ratio bias, thereby favoring mutations that convert XX zygotes into functional males. Maternal sex determination can also evolve as a way to create female biases, in structured populations where brother-sister matings are common and parents are (as a consequence) selected to reduce their investment in sons (Hamilton 1967, 1979; Bull 1983).

Male recombination also must have been suppressed early in the evolution of coccoid PGE, if not prior to its first appearance. It is incompatible with PGE because it creates chromosomes that are mosaics of maternally and paternally inherited segments. An eliminated chromosome would therefore be both paternal and maternal, in parts, and PGE at one locus would entail "MGE" at others (see Haig and Grafen 1991). Haig (1993a) has proposed a model for the evolution of lecanoid PGE, in which male recombination is suppressed as maternally inherited autosomal alleles "join" an already successfully driving maternal X chromosome. The model thereby derives both maternal sex determination and suppressed male recombination from a single primary event (X-chromosome meiotic drive). This is an attractive feature of the model, although suppressed male recombination (achiasmate meiosis) has evolved in many insect taxa, for reasons that remain obscure. Most of these taxa have conventional heterogametic (XO or XY) sex determination (White 1973; Bell 1982).

4.5 Conflicts of Interest in the Evolution of Coccoid PGE Systems

Coccoid PGE probably arose in the Mesozoic (Fig. 3, transition 2), so modern lecanoid systems may share many subsequently derived features that were not part of the first system. The mutation giving rise to the first system might well



have been expressed only in the male germ line, just prior to meiosis, causing meiotic elimination of the paternal chromosome set. This mutation would have enjoyed an enormous transmission advantage, as explained above, and as first noted by Brown (1963, 1964), who called the resulting sweep to fixation an "automatic frequency response". In effect, PGE is a form of conditional (imprint-cued) meiotic drive. Like other driving genes, a maternally inherited PGE allele gains its transmission advantage at the expense of a victim (the

Fig. 3. A phylogenetic hypothesis for some major events in the evolution of coccoid PGE. The tree represents an informal synthesis of ideas from various published and unpublished sources, and should not be considered authoritative. Chromosome systems are keyed at the *lower left* and indicated after the name of each terminal taxon. Kosztarab and Kozár (1988) and Miller (1990) review coccoid evolution and systematics. Several poorly known families have been omitted for the sake of simplicity. Transitions between chromosome systems were inferred by *MacClade* (Maddison and Maddison 1992). The Diaspididae are represented by four lower-level clades that each include both Comstockiella and diaspidid elimination systems, to illustrate the hypothesis that diaspidid PGE has evolved several times; other tribes and genera (apparently monomorphic for one system or the other) are omitted. Basal taxa and the outgroup (aphids) have conventional XX–XO systems with chiasmate meiosis in males, but a 2N-2N system has evolved in *Orthezia* (transition *1*), possibly representing an instance of the driving-X scenario proposed by Haig (1993a). *Lecanoid system.* Late germline elimination is inferred to have arisen once (transition *2*), presumably from a 2N-2N ancestor. The Pseudococcidae (mealybugs) have been well studied, and except for the genus *Puto*, all nonparthenogenic species have lecanoid PGE. *Puto* is conventionally classified as a pseudococcid; it is shown here as basal, in keeping with the hypothesis that its XX-XO system (Brown and Cleveland 1968) is a relic of the ancestral condition for coccoids, not a rederivation (see Nur 1980). *Comstockiella system.* Partial premeiotic elimination is inferred to have arisen near the base of the clade including the remaining ("higher") coccoids (transition *3*); note that Comstockiella systems occur throughout this clade. Lecanoid systems occur sporadically in several of these families and are assumed to represent multiple losses of premeiotic elimination, as discussed in the text. Families are coded as Comstockiella if any of the included species is known to exhibit this system. Very few species of Kerriidae and Cerococcidae have been studied cytologically; these families are coded as lecanoid (transitions *4*), but further study might show that they, too, include Comstockiella species. Kermesidae are coded HET because male somatic cells contain a heterochromatic chromosome set, but spermatogenesis has not been studied (see Nur 1980). *"Breakout" species.* The 2N-2N systems of *Lachnodius* and *Stictococcus* (transitions *5*) appear to represent independent losses of PGE. Male meiosis is achiasmate in both genera (Brown 1977; Brown and Chandra 1977). Unfortunately, no detailed accounts of spermatogenesis yet exist for either of these 2N-2N lineages (see Nur 1980), so there remains a possibility that the cytology of one or both has been misinterpreted. *Multiple origins of the diaspidid system.* The Comstockiella system is primitive for Diaspididae. Three genera in the tribes Odonaspidini and Aspidotini each contain at least one species with the Comstockiella system and at least one with the diaspidid system, and there are Comstockiella-system and diaspidid-system genera in the tribe Diaspidini. This implies that there have been at least four independent origins of the diaspidid system (transitions *6*), and possibly more (Nur 1990a)

paternally inherited homologue), which therefore comes under strong selection to resist being eliminated.

How might such resistance be achieved? There are several possible routes, each of which may have been taken in different lineages and at different times. First, paternally inherited genes could completely block PGE, restoring a fair (but possibly achiasmate) meiosis, followed by functional spermiogenesis for all four meiotic products. One problem with this route is that orthodox male meiosis and spermiogenesis will not have occurred for many millions of years in a typical lineage with PGE, and so during this time some necessary genes may have been lost (see Nur 1970). Second, a single paternal chromosome could escape into the sperm-destined (otherwise maternal) haploid chromosome set, either by exchanging places with its maternal homologue, or by

restoring a random Mendelian segregation of that particular homologue pair. Simply to become euchromatic and join the maternal set in the sperm (like a B chromosome) would be futile, because doing so would cause aneuploidy. Third, paternal genes could somehow cause the "eliminated" paternal sets to undergo spermiogenesis, rather than disintegrating, so that the male's pool of mature sperm would contain at least some all-paternal sperms along with the usual complement of all-maternal sperms.

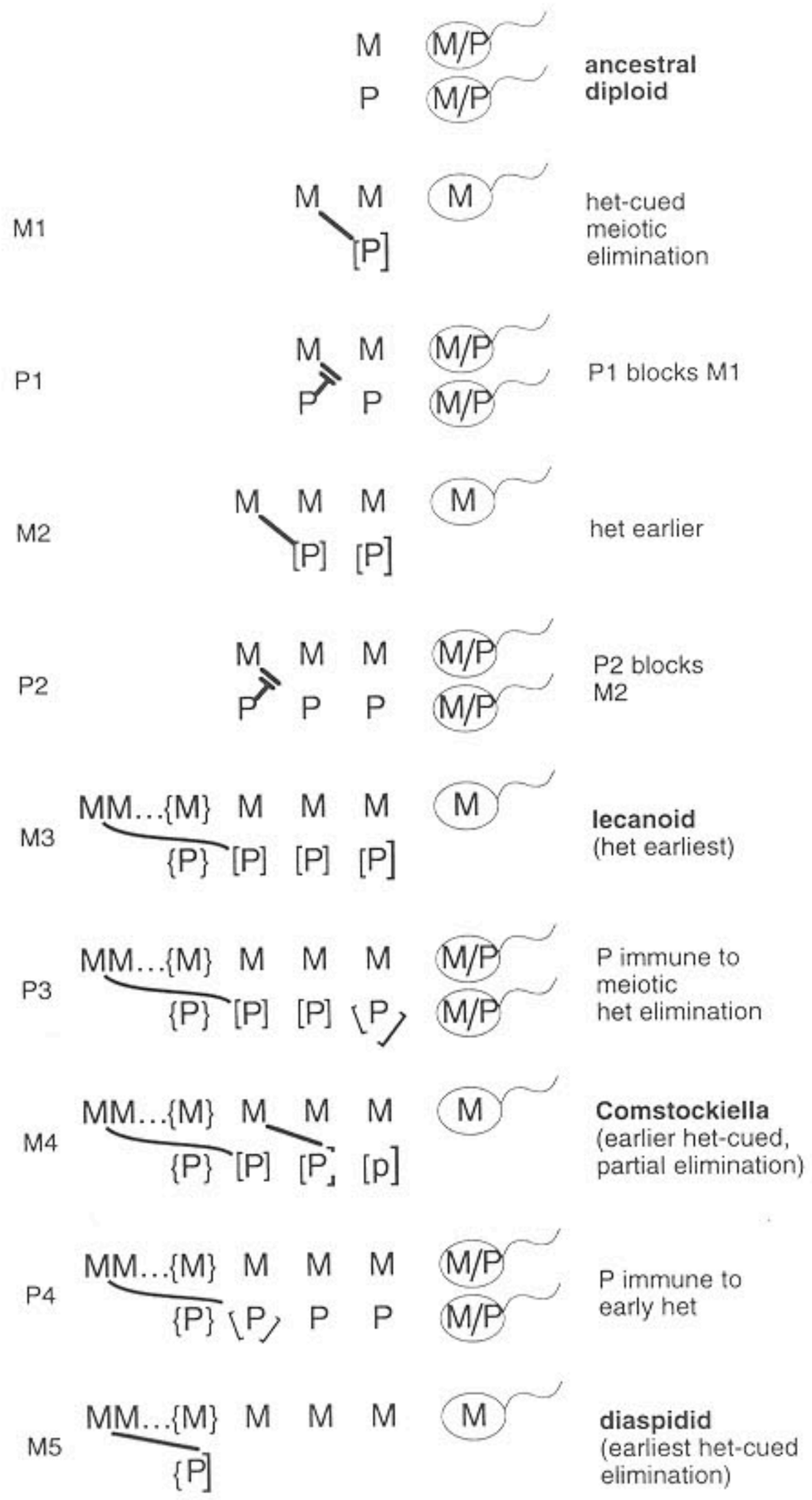
Where and when might such resistance be effected? Because meiotic elimination takes place late in germline development, some paternal resistance genes also might be expressed in the germ line (or at least they might have been, during the early evolution of PGE, before the modern pattern of early lecanoid inactivation had been established). Paternal genes in somatic tissues (of the testis, as well as other parts of the body) might also join the resistance. For example, they could produce diffusible factors (such as hormones) that infiltrate germ cells, affecting chromatin states, meiotic processes, or spermiogenesis. Paternal chromosomes are heterochromatic in both the soma and the germ line of males with modern lecanoid and Comstockiella systems (presumably reflecting maternal suppression of paternal "breakouts", as discussed further below), and heterochromatization is clearly controlled, at least in large part, by genes expressed from the maternally inherited chromosomes (Nur 1990a). However, heterochromatic states can be unstable. For example, in some lecanoid genetic backgrounds the paternal chromosomes tend not to remain fully condensed in meiotic prophase (Nur and Brett 1988), and in many taxa heterochromatic states are reversed in some somatic tissues (Nur 1967, 1980, 1990b; Brown and Chandra 1977), possibly to restore benefits of heterozygosity that are unavailable to cells in which the paternal genome is silenced. Such decondensations pose a danger to maternal interests, since they invite paternal counter moves.

An anti-PGE "counter mutation" that somehow causes a breakout need not be fully effective (or even very effective) to increase rapidly in frequency and go to fixation, because an allele that is occasionally transmitted (when paternally inherited) is much fitter than one that is never transmitted (when paternal). Likewise, even a modest transmission of paternal chromosomes will select strongly for maternal counter-counter mutations that squelch the partial breakout or "leak", since the leak inflicts a fitness loss on maternally inherited genes in those males in which it occurs. An alternating succession of maternally expressed PGE mutations and paternally expressed anti-PGE counter mutations might therefore arise and fix. We suggest that such an evolutionary "struggle" between maternal and paternal genes drove the evolution of coccoid PGE systems. In this model (illustrated schematically in Fig. 4), the partial premeiotic eliminations that occur in Comstockiella systems, and the complete embryonic eliminations that occur in diaspidid systems, are both interpreted as advanced maternal answering moves to paternal counter moves that resulted in full or partial breakouts.

The scenario begins with a maternally expressed PGE mutation (M1) that causes meiotic elimination of the paternal chromosome set, perhaps by making it heterochromatic at the onset of meiosis (see Section 4.3, above, and Fig. 2). This maternal move elicits paternally expressed anti-PGE counter moves that restore a fair meiosis (P1). Such counter mutations might arise frequently, because all paternal genes suffer the same twofold loss of fitness. A successful anti-PGE mutation is likely to act earlier in germline development than the eliminator gene whose effect it blocks, because once the paternal set is heterochromatized (to cause its elimination), it is also silenced. The spread of such an anti-PGE counter mutation selects, in turn, for maternally expressed counter-counter mutations that act even earlier, and so on (M2).

At first, maternally inherited genes would have caused PGE without inactivating paternal genes in the soma, but as more paternally inherited genes evolved anti-PGE effects, inactivation of paternal genes in various somatic tissues (and at earlier times in development) would have been required to sustain reliable PGE. A practical limit to this progression toward earlier and more extensive inactivation is reached when the paternal set is made heterochromatic at just the time when it would first begin expression. In lecanoid systems it becomes heterochromatic early in embryonic development, before the germ line is set aside, at the time when transcription is first detected from the zygotic (maternal) genome (Sabour 1972). This is also near the time when, in diaspidid systems, the paternal set is eliminated. According to our model, these temporal associations are not accidental. Paternal genes cannot resist before they are turned on, so there is no selection to impair or eliminate them at an earlier stage of development, at least if they remain turned off later; but if they somehow manage to resist, later in development (P3), then stronger maternal measures (Comstockiella premeiotic elimination, M4, and diaspidid embryonic elimination, M5) may evolve in response.

Male vigor and fitness will necessarily be compromised by the inactivation of an entire chromosome set in most tissues, because this causes males to suffer the effects of deleterious recessive mutations (leaving aside potential issues of dosage compensation). The fact that heterochromatic states are reversed in certain tissues of at least some species (Nur 1967, 1980, 1990b) implies that euchromatic states could be restored in even more tissues. Why are they not? This pattern of widespread inactivation (with reactivation only in particular tissues) could be explained if paternal genes that are now inactivated formerly engaged in successful resistance. Their success could have favored maternally expressed mutations that caused the initial establishment (or restoration) of paternal heterochromatization in those tissues contributing to the resistance, despite a cost to the male's overall fitness, if maternal chromosomes thereby realized a sufficiently increased rate of differential transmission. Interestingly, in at least some lecanoid species, paternal chromosomes are reactivated in testis sheath and cyst cells, and paternal gene expression (presumably



from those tissues) appears to be required for sperm development (Nur 1967, 1990b; Brown and Chandra 1977).

Among species with the Comstockiella variant of the lecanoid system, different numbers of paternal chromosomes (one, several, all but one, or all) are eliminated in the germ line, prior to meiosis. In some species the particular chromosome(s) eliminated may vary from cyst to cyst within an individual testis, and in some the number of chromosomes eliminated also varies from cyst to cyst (Nur 1965, 1980, 1990a; Kitchin 1975). This early elimination could represent a maternal counter-counter move (M4) that preempts a paternal counter move (P3) that, when it first arose, occasionally

Fig. 4. A model for the evolution of coccoid PGE systems. Evolutionary time is represented as proceeding from *top to bottom*. Developmental time proceeds *left to right*, toward spermiogenesis. Sperm contain only maternal genes (M) or Mendelian assortments from both parents (M/P). The M- and P-sets are shown at developmental stages beginning at the time of action of the most recent PGE or counter-PGE mutation. Ancestral coccoids had XX-XO sex determination and recombination in males, who were chromosomally and transmissionally diploid (Brown 1964; Brown and Chandra 1977; Nur 1980). As in *Sciara* (Smith-Stocking 1936), males expressed both paternal and maternal genes in many tissues, so deleterious recessive alleles existed at many loci. Sex determination came under facultative maternal control, and male recombination was lost, as discussed in the text. Successive PGE and counter-PGE mutations (M1–M5, P1–P4) then gave rise to the modern lecanoid, Comstockiella and diaspidid elimination systems. *Lecanoid system.* An initial PGE mutation (M1) eliminates some or all paternal chromosomes in meiosis II, perhaps by heterochromatizing the P-set in meiotic prophase. Heterochromatic P-sets ([P]) are shown with enlarged closing brackets (]) in the stage where they are eliminated. A counter-PGE mutation (P1), expressed from the P-set earlier than M1 (and possibly in tissues other than the germ line), blocks the effect of M1 and restores full or partial transmission of the P-set. M2 then arises, causing earlier heterochromatization of the P-set (possibly in tissues other than the germ line), suppressing P1 and restoring efficient PGE. Analogues of M2 that act even earlier (and affect even more of the soma) may also arise, but they expose their male carriers to the effects of more deleterious recessive mutations expressed from the M-set in tissues where the P-set is inactivated. By acting only slightly earlier than P1 (and only in relevant tissues), M2 exposes relatively few such mutations and is therefore more easily fixed. Recessive mutations are gradually removed from the gene pool as successively earlier-acting (and more widely-acting) M2-like (and P1-like) mutations fix. In lecanoid males, the P-set becomes heterochromatic at the same time that it would first be expressed (and the M-set is first expressed), as the cleavage nuclei migrate to the periphery of the embryo (Sabour 1972). Both sets are shown as inactive prior to this time ({M}{P}). The earliest-acting lecanoid mutations (M3-like) are expressed not from the M-set, but from the diploid maternal genome during oogenesis. *Comstockiella and diaspidid systems.* A counter-mutation (P3) allows the meiotic P-set to become euchromatic and escape elimination. M4 then causes premeiotic (Comstockiella) elimination of some paternal chromosomes, facilitating a reestablishment of heterochromatization of the remainder (p) and their lecanoid elimination in meiosis II. Another counter-mutation (P4) appears and fixes, allowing the P-set to escape later elimination in meiotic prophase or meiosis II (possibly by becoming euchromatic prior to meiotic prophase). Because deleterious recessive mutations are now quite rare (owing to the functionally hemizygous state that prevails in most tissues), the diaspidid mutation (M5) is a viable answer to P4. Expressed from the diploid maternal genome in oogenesis, it causes destruction of the P-set at the end of the rapid cleavages, before the germ line is set aside

spared at least some paternal chromosomes from elimination, by causing them to decondense and thereby compete with their maternal homologs for membership in the sperm-destined chromosome set. A partial premeiotic (Comstockiella) elimination might help to block a paternal escape, if a reduced number of germline paternal chromosomes experienced an effectively higher concentration of some maternally expressed substance needed to keep them securely heterochromatic, silent, and clumped at critical stages of meiosis. If the efficiency of PGE were increased as a consequence, then the genes causing early partial elimination could increase in frequency under

selection. Comstockiella elimination also could have evolved to thwart the escape of entire paternal chromosome sets from lecanoid elimination (see below).

Embryonic elimination (M5) represents a decisive maternal move, because no answering paternal counter move is possible in a male with no paternal chromosomes. Mechanistic (developmental) obstacles might often slow or prevent the evolution of embryonic elimination, but it should evolve relatively easily in lineages with histories of embryonic paternal gene inactivation, where male development and physiology will have had time to adapt, gradually, to an effectively haploid somatic genome (as discussed in the legend to Fig. 4).

In 1963, Brown proposed an amazingly modern move-countermove scenario to explain evolutionary transitions between lecanoid and Comstockiella systems. In this model, some mutations go rapidly to fixation by "automatic frequency response"; others that counteract the effects of these previously fixed mutations then arise and fix; and so on. Unfortunately, Brown's model makes two debilitating assumptions. First, it assumes (incorrectly) that when paternal chromosomes seem to disappear in Comstockiella meiotic prophase, they merely become *invisible* and will, in fact, be *transmitted* in sperm. Thus the model seeks to explain a nonevent. Second, the model assumes that paternal chromosomes are heterochromatic in *all* male tissues, and that "fertility factors" therefore must be expressed from such heterochromatic paternal chromosomes (Nelson-Rees 1962). A few years later, Nur (1967) showed that paternal chromosomes become euchromatic and genetically active in some tissues of the testis, thereby explaining more plausibly why the sons of heavily X-irradiated male mealybugs are usually infertile. Although badly misled by these two assumptions, Brown clearly saw that PGE involves conflicts of interest. Had his life not ended tragically at the height of his career (Dempster et al. 1978), he surely would have constructed a successful model.

4.6 Evidence of "Breakouts"

A paternal counter mutation that fully restores diplodiploidy to a population with a prior history of PGE thereby creates a fully diploid species that is embedded phylogenetically within a clade for which PGE is clearly the ancestral condition. Two such apparently revertant (2N-2N) taxa are known in Coccoidea (Nur 1980; transitions 5 in Fig. 3), and our model clearly predicts that others should eventually be found. Of course, such revertants might often be short-lived, since new maternal mutations that restore PGE might well arise.

Do functional sperm ever develop from heterochromatic paternal sets? Uzi Nur (pers. comm.) notes that electron micrographic sections through mature sperm cysts of lecanoid- and Comstockiella-system males occasionally show more than the expected number of sperms (e.g., 17 or 18 instead of 16, in species where 16 is the standard number; see Robison 1990, Fig. 8, for an example). Molecular techniques could be used to screen thousands of sperm or hundreds of offspring at a time for rare instances of paternal allelic transmission (whether by this route or any other).

A partial premeiotic (Comstockiella) elimination could thwart this kind of breakout by making the paternal sets aneuploid. However, this maternal move is unlikely to succeed, over the long run, unless the development of aneuploid spermatids is usually blocked at some later stage. Such a block might well evolve, because every aneuploid sperm that fertilizes an egg wastes a potentially viable zygote. Both maternally and paternally inherited genes stand to benefit from a mechanism that reduces such wastage. Given a mechanism that detects and destroys aneuploid spermatids, the Comstockiella strategy should be relatively simple to implement, and highly effective.

One possible paternal response to this maternal strategy would be to convert the Comstockiella system back to a (leaky) lecanoid system, in which euploid (but heterochromatic) paternal sets are generated in meiosis. In Comstockiella systems where variable numbers of paternal chromosomes are eliminated at meiotic prophase, as few as zero are eliminated in some cysts (Nur 1965; Kitchin 1975), in which "spermatogenesis resembles that of the lecanoid system" (Nur 1990a). There are also some apparently fully lecanoid species (zero in all cysts) with Comstockiella ancestors (Fig. 3). This variation can be interpreted as one reflection of an ongoing evolutionary struggle between paternally expressed breakout mutations and maternally expressed suppressors of such mutations.

The distribution of Comstockiella and diaspidid systems within the Diaspididae strongly implies that diaspidid systems arose on at least four (and probably six or more) occasions from Comstockiella systems, which appear to be primitive for Diaspididae (Nur 1980, 1990a; transitions 6, Fig. 3). The alternative possibility is that Comstockiella systems have been re-derived on several occasions from an ancestral diaspidid embryonic-elimination system, but this seems less likely on mechanistic grounds, since it demands the reactivation of a complex developmental program for germline behaviors that would have been unused for significant evolutionary time (see Bull and Charnov 1985). If diaspidid elimination evolves to squelch paternal leaks, then we might expect to find that diaspidid-system lineages tend to be closely related to relatively leaky Comstockiella-system lineages.

5 PGE and Arrhenotoky in Mites and Bark Beetles

The commonness of arrhenotoky relative to embryonic PGE may be overestimated for animals as a whole, because in many taxa the evidence for arrhenotoky consists largely of haploid chromosome numbers in adult male

somas (see Bell 1982; Norton et al. 1993). In relatively few cases are there unequivocal demonstrations that unmated females produce normal males, and only rarely are there the careful cytogenetic studies needed to detect embryonic PGE (which often occurs inside the mother). In some taxa where males are known to be haploid and where unmated females are known not to produce sons, the genetic system has been described as "arrhenotokous gynogenesis", with the implication that fertilization is required to stimulate male development, although paternal chromosomes play no continuing role. Without a cytological study of early embryos, this hypothesis cannot be distinguished from that of embryonic PGE. Thus some taxa presently characterized as arrhenotokous are undoubtedly pseudoarrhenotokous. For example, arrhenotoky is widespread in mites, but the phytoseiid mites appear to be largely pseudoarrhenotokous, with an embryonic PGE system similar in many ways to the coccoid diaspidid system (see Treat 1965; Nelson-Rees et al. 1980; Schulten 1985; Norton et al. 1993; Sabelis and Nagelkerke 1993).

The beetle family Scolytidae has long been known to include many arrhenotokous species (reviewed by Kirkendall 1993). One member of this family, the coffee berry borer *Hypothenemus hampei*, recently has been shown to be pseudoarrhenotokous, with germline elimination that bears striking similarities to the lecanoid system (Brun et al. 1995; Borsa and Coustau 1996; Borsa and Kjellberg 1996). In somatic cells the paternal chromosome set appears "compacted into a darkly staining ball of chromatin", and during the single meiotic division in spermatogenesis "the paternally derived set begins to degenerate while the maternally derived set condenses and divides" (Brun et al. 1995). This remarkable discovery will undoubtedly stimulate a search for other pseudoarrhenotokous scolytids that have been misclassified as arrhenotokous. The family includes species with diploid, haplodiploid, and (now) parahaplodiploid genetic systems, and these systems seem to be correlated with aspects of ecology and population structure (Kirkendall 1993), so the scolytids may present unusually good opportunities to reconstruct the evolutionary histories of PGE systems. They may also present opportunities to compare independently derived PGE mechanisms, both within the family and between the family and other groups such as coccoids.

6
PSR and *Wolbachia* as Direct-acting Eliminators

In the wasp *Nasonia vitripennis*, a zygote's paternal chromosomes may be eliminated by either of two mechanisms that act at the first (gonomeric) mitosis. The first mechanism depends on a B chromosome called *PSR*, for paternal sex ratio (Nur et al. 1988). When a sperm from a male carrying *PSR* fertilizes an egg, the paternal genome (except for *PSR*) fails to complete the first mitosis and is lost by anaphase lag; but *Nasonia* is haplodiploid, like most Hymenoptera, so the resulting haploid embryo develops as a normal male. The

PSR chromosome escapes the destruction inflicted on the paternal set, so an embryo that would otherwise have been diploid and female turns into a haploid male carrying *PSR*. This "ultra-selfish" strategy is expected to maintain *PSR* at high frequencies only in populations where females fertilize more than 50% of their eggs. *Nasonia* females do this (as predicted) in situations where their daughters and sons are likely to mate with each other (see Werren 1991).

The second PGE mechanism of *Nasonia* is effected by intracellular parasitic bacteria of the genus *Wolbachia*. These bacteria cause cytoplasmic incompatibility by interfering with paternal chromosome segregation in the gonomeric mitosis in embryos whose parents are infected with *Wolbachia* of incompatible genotypes, or whose mother is *not* (but father *is*) infected. In many other insects, including *Drosophila*, *Wolbachia* also destroy chromosomes (presumably paternal ones), and the affected embryos die (reviewed by O'Neill et al. 1992, 1997; Rousset et al. 1992; Moran and Baumann 1994; Werren et al. 1995; Lassy and Karr 1996; Werren 1997). In *Nasonia* the paternal genome fails in the same way and at the same stage of development; but instead of killing the female-destined embryo, this elimination converts it to a viable haploid male. *Wolbachia* are transmitted through the egg, not the sperm, so a seemingly "spiteful" conversion of females to males can increase the local frequency of *Wolbachia*-infected females by reducing the frequency of uninfected females (Hurst 1991; Hurst et al. 1997; see Werren 1997). When an infected male mates with a female infected by the same strain of *Wolbachia*, the zygote's paternal chromosomes survive (as in *Drosophila*), indicating that the *Wolbachia*-infected egg cytoplasm provides a substance that rescues the paternal chromosomes from destruction (see Lassy and Karr 1996; Werren 1997).

These two mechanisms of PGE differ in that one involves a supernumerary chromosome (*PSR*), while the other involves infection by a microorganism (*Wolbachia*). In addition, they can exist and function separately or together (Dobson and Tanouye 1996). In incompatible crosses, *Wolbachia* causes the destruction of *PSR* along with the rest of the paternal chromosome set, so *PSR*'s immunity to its own effect does not protect it from the effects of *Wolbachia* (Dobson and Tanouye 1996). However, despite these differences, the two mechanisms of PGE share striking genetic and cytological similarities, suggesting some deeper connection including (perhaps) related origins (Reed and Werren 1995). For example, *PSR* may have arisen from one or several paternal chromosome fragments generated during a cytoplasmic-incompatibility reaction caused by *Wolbachia* (Ryan et al. 1985; Nur et al. 1988; Reed 1993; Reed et al. 1994).

Do *PSR* and *Wolbachia* "imprint" the chromosomes they manipulate? The literature on these systems often invokes imprints, laid down in the male by *PSR* or *Wolbachia*; but these eliminations do not obviously involve chromosomal marks that are *replicated* along with the chromosomes. Some sort of "lesion" must be created during spermatogensis, and it must *persist* through

one round of replication, but it need *not* be *replicated*. Presumably, a compatible *Wolbachia*-infected egg cytoplasm provides a substance that "repairs" the "lesion" that otherwise would lead to destruction of the paternal chromosomes. In a male that carries *PSR*, similar lesions could be created during spermatogenesis, with similar consequences in the zygote. Interpretations of *PSR*'s effects are complicated, however, by its physical presence in the affected mitosis.

In short, the paternal genome eliminations induced by *PSR* and *Wolbachia* can be explained by direct effects of these selfish chromosome-manipulating parasites on the chromosomes to be eliminated. There is no need (yet) to postulate a heritable epigenetic mark that would have no effect unless it were read by a mechanism that responded to the information contained in the mark. The purpose of this cautious interpretation is to focus attention on the essential features of Metz's original concept and definition of imprinting. We do not claim that an involvement of imprints in these systems can be ruled out, but merely that none has yet been established. Indeed, as mentioned above, we are intrigued by the possibility that systems of direct PGE, such as these, may open windows into the early evolution of at least some forms of imprint-mediated PGE.

7
Perspective on Imprints and Conflicts of Interest

We have emphasized the relationships between imprints and the mechanisms that use them as cues, but why do imprints exist? Some could be byproducts of gametogenesis. Others could have evolved to provide parent-of-origin information used to direct development in ways that benefit the entire organism and all its genes (as is assumed, uncritically, by many biologists). In either case, imprints would inevitably be, in addition, a source of information that could be exploited by mechanisms that implement selfish strategies driven by intragenomic conflicts of interest. Of course, some imprints may have evolved solely to inform genes that act out such conflicts. However, in that case they might well be opposed by genes elsewhere in the genome that never stand to benefit from the conflict.

Maternal and paternal contributions to the zygote are highly unequal, and these underscore the abundant opportunities for conflict that exist in eukaryotic development. Except for the pronuclei, most components of the zygote are derived primarily from one gamete or the other. The egg provides maternal mRNAs and diverse nutrients, and although paternal mitochondria are introduced from the sperm, maternal mitochondrial DNAs dominate numerically and paternal ones are usually destroyed (see Cosmides and Tooby 1981; Avise 1994; Meusel and Mortiz 1993; Godelle and Rebold 1995; Kaneda et al. 1995; Hurst et al. 1997; Pitnick and Karr 1998); likewise, intracellular bacteria such as *Wolbachia* are transmitted in the egg, not the sperm.

However, paternal factors can be introduced with or within the sperm (Agulnik et al. 1993; Yasuda et al. 1995; Browning and Strome 1996; Herrera et al. 1996; reviewed by Karr 1996). For example, in most animals (including humans) the sperm provides centriolar functions (minus certain components provided by the egg) as proposed by Boveri nearly a century ago (reviewed by Schatten 1994; Gall 1996; Sathananthan 1997). Mice (and possibly rodents generally) are exceptional, in that the egg provides the centriole. Schatten notes that the requirement for both paternal and maternal components to reconstitute zygotic centriolar function should prevent parthenogenesis, and he suggests that this may be the purpose of this system. He proposes that the exception to Boveri's rule in mice reflects an evolutionary replacement of this system with one of complementary (maternal and paternal) genomic imprints. Others have proposed that imprinting evolved to prevent parthenogenesis and ovarian tumors, on the grounds that imprinting lacks any other obvious adaptive function (reviewed and elaborated by Varmuza and Mann 1994a). This proposal has been highly controversial (Haig 1994; Moore 1994; Solter 1994; Varmuza and Mann 1994b; Haig and Trivers 1995), but it serves to remind us that the function(s) of imprinting remain frustratingly obscure. Is "imprinting" a misleadingly simple name for diverse phenomena with diverse causes? Might the various uniparentally contributed zygotic components represent just a few of many distinct sources of conflict that drive the evolution of imprint-cued phenomena such as paternal gene exclusion and differential gene inactivation?

Acknowledgments. We thank J. S. Burner, A. Burt, E. L. Charnov, B. de Saint Phalle, W. J. Dickinson, K. Golic, D. Haig, P. G. Johnston, T. L. Karr, S. Mango, D. L. Miller, N. A. Moran, U. Nur, S. L. O'Neill, M. H. Richards, H. Robertson, J. R. Roth, W. Sullivan, R. Trivers, and J. H. Werren for stimulating discussions, comments on the manuscript, and other forms of help. We dedicate this chapter to Uzi Nur, with gratitude for his teaching, and with deep admiration for his many extremely important contributions to genetics.

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