THE ROYAL SOCIETY

Origin of a haplodiploid beetle lineage

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The beetle family Scolytidae includes several groups having regular sib-mating and extremely female-biased sex ratios. Two such groups are known to include haplodiploid species: (i) the tribe Xyleborini and (ii) *Coccotrypes* and related genera within the tribe Dryocoetini. Relationships of these groups have been controversial. We analysed elongation factor 1-α (852 bp) and cytochrome oxidase 1 (1179 bp) sequences for 40 species. The most-parsimonious trees imply a single origin of haplodiploidy uniting Xyleborini (approximately 1200 species) and sib-mating Dryocoetini (approximately 160 species). The sister-group of the haplodiploid clade is the outcrossing genus *Dryocoetes*. The controversial genus *Premnobius* is outside the haplodiploid clade. Most haplodiploid scolytids exploit novel resources, ambrosia fungi or seeds, but a few have the ancestral habit of feeding on phloem. Thus, scolytids provide the clearest example of W. D. Hamilton's scenario for the evolution of haplodiploidy (life under bark leading to inbreeding and hence to female-biased sex ratios through haplodiploidy) and now constitute a unique opportunity to study diplo-diploid and haplodiploid sister-lineages in a shared ancestral habitat. There is some evidence of sex determination by maternally inherited endosymbiotic bacteria, which may explain the consistency with which female-biased sex ratios and close inbreeding have been maintained.

Keywords: inbreeding; sex ratio; Coleoptera; sib-mating; Scolytidae; haplodiploidy

1. INTRODUCTION

One of the most dramatic recurring genetic system transformations seen in animals is the origin of haplodiploidy, a system in which males develop from unfertilized eggs and are haploid. There are now thought to have been at least 17 origins of haplodiploidy in Metazoa, 15 of these in arthropods (Mable & Otto 1998). Most of these origins are poorly resolved phylogenetically—neither basal haplodiploid lineages nor diplodiploid sister lineages have been identified (Beutel & Haas 1996; Blaxter *et al*. 1998; Bull 1983; Crespi *et al*. 1996; Garey *et al*. 1996; Hoernschemeyer 1998; Norton *et al*. 1993; Nur 1980; Von Dohlen & Moran 1995; White 1973; Whiting *et al*. 1997)—preventing an understanding of the ecological, demographic, and cytogenetic context in which haplodiploidy arose.

Hamilton (1967) suggested that most origins of haplodiploidy in arthropods have occurred under conditions of extreme inbreeding. Highly female-biased brood sex ratios are strongly favoured under inbreeding, and haplodiploidy provides a means of sex ratio adjustment. Hamilton (1978) further suggested that the habitat in which haplodiploidy has arisen most often is under the bark of dead trees. Most of the haplodiploid taxa cited to support these hypotheses are ecologically diversified groups in which the ancestral habit is unclear: Hymenoptera (Vilhelmsen 1997; Whitfield 1998; Whiting *et al*. 1997), Thysanoptera (Crespi *et al*. 1996; Von Dohlen & Moran 1995), and the mite clades Dermanyssina and Eleutherengona (Norton *et al*. 1993). Indeed there is just one group cited by Hamilton in which all of the haplodiploid lineages appear to be inbreeding, and almost all live in dead wood: the beetle family Scolytidae (Kirkendall 1993). This conservativeness in the habits of scolytids make it likely that close phylogenetic and comparative studies could yield an unusually clear picture of the origin or origins of haplodiploidy in this group. In this paper we present the results of a phylogenetic investigation of the origins of haplodiploidy in the Scolytidae.

Seven different tribes of scolytids include inbreeding species, and five of these (Xyleborini, Dryocoetini, Cryphalini, Corthylini and Hyorrhynchini) include species with dwarfed, flightless males and extremely female-biased sex ratios (Kirkendall 1993), exemplifying Hamilton's (1967) 'biofacies of extreme inbreeding and arrhenotoky'. For an inbreeding species in one of these tribes (Cryphalini), males have now been found to be diploid, but to have a paternal genome that is not expressed and is eliminated in spermatogenesis (Brun *et al*. 1995), a system sometimes termed pseudoarrhenotoky or paternal genome elimination (PGE) (Herrick & Seger 1999). For the inbreeders in two other tribes (Corthylini and Hyorrhynchini) nothing is known of the cytogenetic mechanism of male production. However, for inbreeders in the tribes Xyleborini and Dryocoetini, true haplodiploidy (arrhenotoky, in which unfertilized eggs develop into haploid males) has been shown (Kirkendall 1993).

This study focuses on the origin of true haplodiploidy in the tribes Xyleborini and Dryocoetini. Extreme inbreeding characterizes the entire tribe Xyleborini and a group of genera within the tribe Dryocoetini (*Coccotrypes*, *Dryocoetiops*, *Ozopemon* and a genus to be described by Bright & Rabaglia (1999)). All of the species in these two groups have highly female-biased sex ratios, and the

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Table 1. *Primers used for PCR amplification and sequencing*

locus	name[a]	alias	use[b]	sequence[c] (5′ to 3′)
co1	s1541	Zeus	p, c	tgakcyggaatastaggaicatc
co1	s1847		c	ggagcaggaacaggttgaac
co1	s2215	s2191, rcNancy	c	cccggtaaaattaaaatataaacttc
co1	s2213		c	cctgaagtttayattttaattytncc
co1	s2617		c	gcacactttcactatgttctatcaatag
co1	a2167		c	cttcgggatgtccaaaaaatc
co1	a2237		c	ccgaatgcttctttttttacctctttcttg
co1	a2411	a2442, rcDick	p, c	gctaatcatctaaaaactttaattccwgtwg
co1	a2590		p, c	gctcctattgatarwacatartgraaatg
co1	a2596		p, c	gctcctatwganarwacatartg
co1	a2771		p, c	ggatartcagartaacgtcgwggtatwc
co1	a2940	a2963, rcJesse	p, c	aggaggttcattataigaatgttc
ef-1α	efs149	efs149b	p, c	atcgagaagttcgagaaggaggcycargaaatggg
ef-1α	efs466	efs-I	p, c	gtcggtgtcaacaaaatgg
ef-1α	efs562	efs-II	c	ggttacaatccngctgctg
ef-1α	efs701	efs-III	c	ctcttattgaygctttggatgc
ef-1α	efa544	efa-II	c	cagcagcnggattgtaacc
ef-1α	efa680	efa-III	c	gcatccaaagcrtcaataagag
ef-1α	efa692	efa-IV	p, c	ggtgggagaatrgcrtccaaag
ef-1α	efa754	efa-V	p, c	ccaccaattttgtagacatc
ef-1α	efa923	efa923	p, c	acgttcttcacgttgaarccaa
ef-1α	efa1043	efa-1106	p, c	gtatatccattggaaatttgaccnggrtgrtt

[a] Names refer to direction (s, sense; a, antisense) and to the position of the 3′-end. For ef-1α (elongation factor 1-α), names refer to position in the coding sequence only, as in Danforth & Ji (1998); for mitochondrial DNA (mtDNA), names refer to position in the mtDNA genome, as in Simon *et al.* (1994). S1541 was designed by B. Crespi (Simon Fraser University). We also used, for both amplification and sequencing, the following co1 (cytochrome oxidase 1) primers (Simon *et al.* 1994): C1-J-1718, C1-J-2183, C1-J-2441.
[b] p, PCR amplification; c, sequencing cycle.
[c] IUPAC ambiguity codes (Cornish-Bowden 1985) refer to equal mixtures of bases; i, inosine.

males that have been described from them are typically dwarfed and invariably flightless (Kirkendall 1993). The precise sex ratio control widespread in Xyleborini and inbreeding Dryocoetini (often one male per brood) is in itself usually indicative of haplodiploidy (Hamilton 1967), and in both Dryocoetini and Xyleborini, haplodiploidy has been experimentally confirmed for a few species (Kirkendall 1993; Ueda 1997). Though it remains possible that some other unknown sex determination mechanism, one that is similar to haplodiploidy with respect to brood sex ratio control, also exists in Xyleborini or inbreeding Dryocoetini, at present haplodiploidy is the only genetic system known in either group and here we treat both groups as haplodiploid.

Relationships of these groups to each other and to other groups of scolytids have been controversial. In the only comprehensive published hypothesis of the tribal relationships in Scolytidae, Wood (1982) placed Xyloterini as the sister group of Xyleborini and Crypturgini as the sister group of Dryocoetini, implying two independent origins of haplodiploidy. A much closer relationship between Xyleborini and inbreeding Dryocoetini, implying a single origin of haplodiploidy, was suggested by Browne (1959), on the basis of adult morphology, by Lekander (1968), on the basis of larval characters, and by Nobuchi (1969), on the basis of characters of the proventriculus, but none of these authors presented a formal analysis supporting these suggestions or a formal classification reflecting them. A recent review of haplodiploidy draws attention to this lack of agreement regarding the number of origins in Scolytidae (Mable & Otto 1998). To help resolve this question, we have studied the phylogenetic relationships of inbreeding haplodiploid scolytids using nuclear (elongation factor 1-α (ef-1α)) and mitochondrial (cytochrome oxidase 1 (co1)) DNA sequences.

2. MATERIAL AND METHODS

(a) *Taxonomic sampling*

Sampling was focused on the tribes Xyleborini (nine out of the 25 described genera were sampled for this study) and Dryocoetini (three out of the four inbreeding genera and five out of the 15 outbreeding genera), and on tribes thought to be closely related to these (Wood 1982): Xyloterini (two out of the three genera), Crypturgini (two out of the six genera), and Ipini (five out of the six genera). Also included as outgroups are six other genera of Scolytinae, across four tribes not thought to be closely related to the haplodiploid lineages.

(b) *Polymerase chain reaction and sequencing*

We used a polymerase chain reaction (PCR) and automated cycle sequencing to obtain partial sequences of ef-1α (852 bp pairs of coding sequence) and co1 (1179 bp). DNA was extracted according to the 'salting out' protocol of Sunnucks & Hales (1996), as modified in Normark (1999). For PCR reactions (50 µl total volume), the following solution was used: 0.2 µM of each primer (see table 1), 0.8mM dNTPs, Qiagen® (Valencia, CA, USA) PCR buffer with additional $MgCl_2$ to a final concentration of 2.5 M, and 1.25 units Qiagen® Taq DNA polymerase. For co1, the temperature profile consisted of 40 cycles as follows: 95 °C for 30 s, 47 °C for 60 s, and 72 °C for 60 s. For ef-1α, a touchdown profile of 42 cycles was used, with annealing

temperature decreasing from 58 °C to 42 °C by 2° every third cycle, and the final 18 cycles at 42 °C; timings and other temperatures were the same as for col. PCR products were purified using either the Qiagen® gel extraction kit or the Qiagen® PCR purification kit and directly sequenced using the ABI dye terminator cycle-sequencing kit and an ABI 370A automated sequencer (PE Biosystems, CA, USA).

For ef-1α we found evidence of two different loci, as are also seen in *Drosophila* and *Apis* (Danforth & Ji 1998), that differ in the positions of their introns (B. B. Normark, B. H. Jordal and B. D. Farrell, unpublished observations). For this study we used only one of the two putative loci (having one intron in the fragment sequenced, between coding positions 753 and 754). The sequence of the intron itself was omitted from the phylogenetic analysis. The sequences have been submitted to GenBank under accession numbers AF186657–AF186696 (ef-1α) and AF187107–AF187146 (col).

(c) *Phylogenetic analysis*

We used test version 4.0d64 of PAUP* (Swofford 1998) to search for most parsimonious trees, with all substitutions weighted equally. Searches were heuristic, with 100 random-addition-sequence starting trees; for bootstrapping and incongruence testing we used 100 replicates, each with 20 random-addition-sequence starting trees. The ef-1α matrix was analysed on its own and in combination with an amino-acid translation of the col matrix. Four different versions of the col matrix were analysed separately: translated and untranslated, with and without the incongruently placed taxon *Pityogenes hopkinsi*. We tested for incongruence between the two genes, using the partition homogeneity test of Farris *et al.* (1995), as implemented in PAUP*.

(d) *Tests of critical nodes*

To test whether alternative topologies were significantly longer than the most parsimonious trees, we repeated the searches (for the combined matrix and the ef-1α matrix, with all taxa included) with each of the following nodes (Wood 1982, 1986) successively constrained to monophyly: Xyleborini including *Premnobius*, Xyleborini excluding *Premnobius*, Ipini (excluding *Premnobius*), Dryocoetini (excluding Xyleborini), Xyleborini (with and without *Premnobius*) + Xyloterini, Dryocoetini + Crypturgini. Using Templeton's Wilcoxon signed-rank test, and (for ef-1α only) the Kishino–Hasegawa test, as implemented in PAUP*, we compared the shortest constrained trees the globally shortest trees, for each of constraints and each of the data sets.

3. RESULTS

(a) *The combined analysis*

Results of the combined analysis of ef-1α nucleotides and col amino acids are presented in figure 1. There is strong support (bootstrap 100%; decay index 14) for a single clade that includes all the inbreeding Dryocoetini and all but one genus (*Premnobius*) of Xyleborini. All of the species in this clade have highly female-biased sex ratios and are believed to be regularly sib-mating (Kirkendall 1993); the clade includes all the known haplodiploid lineages of scolytids and no lineages known to have any other genetic system, and is hereafter referred to as 'the haplodiploid clade' (see position of arrow in figure 1). There is also strong support (bootstrap 98%; decay index 14) for a sister-group relationship between the haplodiploid clade and the diplodiploid, outbreeding genus *Dryocoetes*.

(b) *Separate analyses of ef-1α and col*

There was significant incongruence between the ef-1α nucleotides and the col amino acids ($p = 0.03$), according to the partition homogeneity test. The most striking topological incongruence between the two separate analyses was in the placement of the diplodiploid, outbreeding ipine species *Pityogenes hopkinsi*. In the most parsimonious trees resulting from the ef-1α analysis, *P. hopkinsi* was nested within the tribe Ipini as expected. In the col results, *P. hopkinsi* nested within the haplodiploid clade. When *P. hopkinsi* was excluded, the partition homogeneity test did not find significant incongruence ($p = 0.16$) and the col analysis recovered the same haplodiploid clade as the ef-1α analysis, and also recovered the same sister-group relationship between *Dryocoetes* and the haplodiploid clade (figure 1).

(c) *Tests of critical nodes*

The Templeton tests and the Kishino–Hasegawa test all permit hypotheses of monophyly to be rejected, at either the $\alpha = 0.01$ (**) or $\alpha = 0.05$ (*) level, for the following clades: Xyleborini including *Premnobius***, Xyloterini + Xyleborini (with** or without* *Premnobius*), Dryocoetini + Crypturgini*. Two of the three tests permitted rejection of a monophyletic Dryocoetini (combined data, Templeton*; ef-1α, Templeton, $p = 0.07$; ef-1α, Kishino–Hasegawa*). None of the tests could reject a monophyletic Xyleborini (excluding *Premnobius*) or Ipini (excluding *Premnobius*).

4. DISCUSSION

(a) *Ecology and diversification of haplodiploid scolytids*

With the results presented here, the origin of haplodiploidy in scolytids is now the best understood out of the 17 known origins of haplodiploidy, in the following sense: the diplodiploid sister lineage of the haplodiploid clade has been identified (the genus *Dryocoetes*); all the species of the sister lineage, and most other phylogenetically proximate outgroups, all share the same habit (adults and larvae both tunnel under the bark of injured or recently killed trees, consuming the phloem); and the same habit is found in some members of the haplodiploid clade (figures 1 and 2). Thus in scolytids it is now possible to compare diplodiploid and haplodiploid sister groups that can both be unambiguously inferred to occupy the same ancestral environment and to have (apart from dispersal and breeding biology) largely the same ancestral habits. In no other case is this now possible (Beutel & Haas 1996; Blaxter *et al.* 1998; Crespi *et al.* 1996; Garey *et al.* 1996; Hoernschemeyer 1998; Mable & Otto 1998; Von Dohlen & Moran 1995; White 1973; Whiting *et al.* 1997), though future phylogenetic analyses may ultimately make it possible in other cases, especially in scale insects and mites (Norton *et al.* 1993; Nur 1980).

The origin of haplodiploidy in Scolytidae was followed by diversification (figure 2), both in terms of the number of species and in terms of the exploitation of new

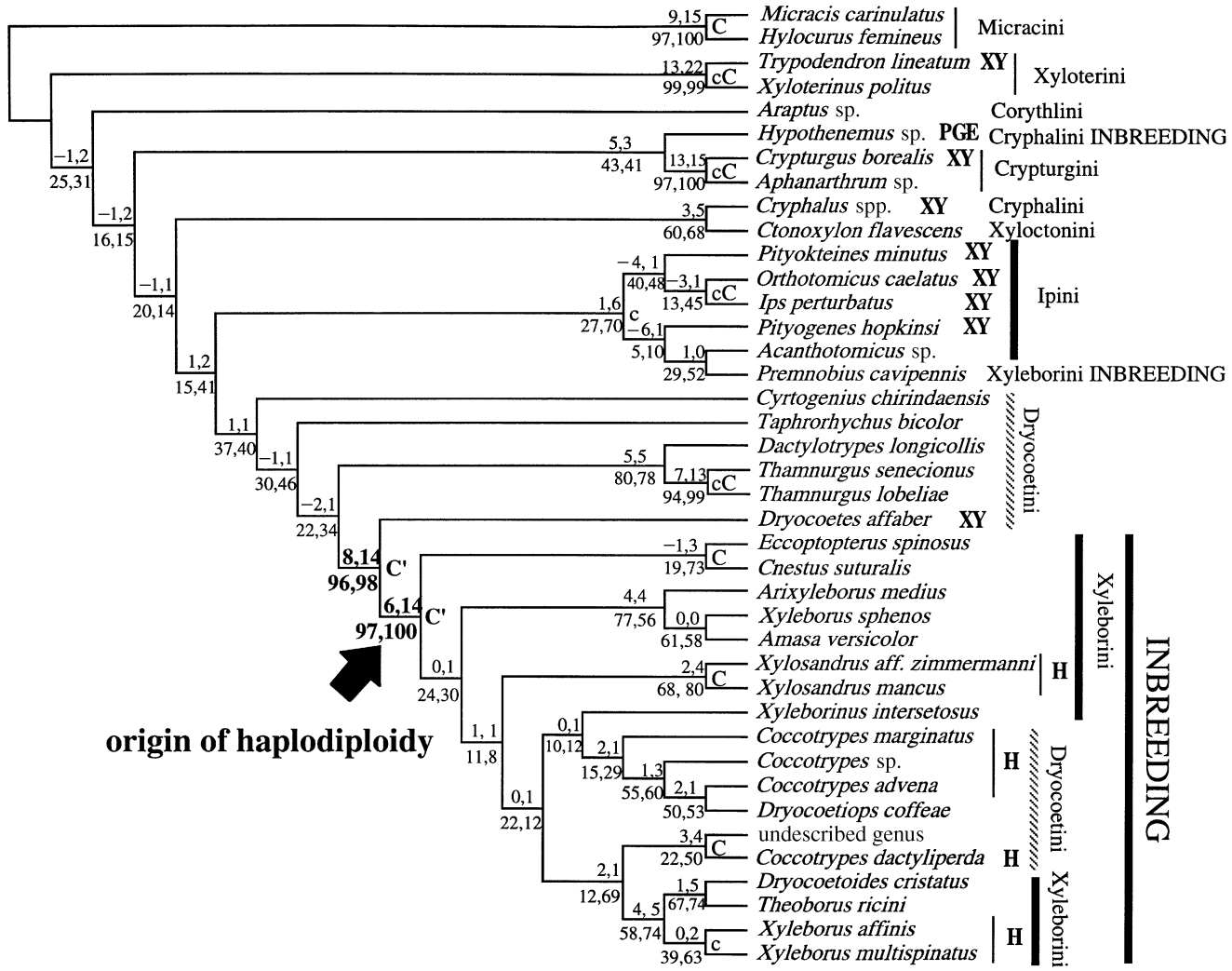

Figure 1. One of four most parsimonious trees from the combined analysis of ef-1α nucleotides and co1 amino acids. Length = 2639; CI (informative characters only) = 0.297; RI = 0.444. Above each internal branch are the decay indices for that branch under the ef-1α nucleotide analysis and the combined analysis, respectively. (A decay index of zero marks a branch that occurs in some but not all most parsimonious trees; a negative decay index marks a branch that does not occur in any most parsimonious trees (for ef-1α) and its absolute value indicates the cost of imposing the branch as a constraint.) Below each internal branch are the bootstrap values for that branch. Support for a branch from analysis of co1 nucleotides (c) or amino acids (C) is indicated to the right of the branch. Branches marked C′ are supported by co1 only if the incongruently placed taxon *Pityogenes hopkinsi* is excluded. Genera in which the genetic system has been investigated (Brun *et al.* 1995; Kirkendall 1993; Smith & Virkki 1978) are marked H (haplodiploid), XY (diplodiploid; males are heterogametic, with X and parachute y sex chromosomes), or PGE. Except within the genus *Ips*, which has a few polyploid, apomictic, pseudogamous lineages not indicated here, no variation is known within genera for the cytogenetic characters reported. Tribal designations follow Wood (1986) and Wood & Bright (1992). Taxa marked INBREEDING have highly female-biased sex ratios and dwarfed, flightless males; all of the other taxa are outbreeding with even sex ratios, flying males, and little size dimorphism. The inferred position of the origin of haplodiploidy is based on the confirmation of haplodiploidy in what are effectively independent basal lineages within the inbreeding clade (given the lack of phylogenetic resolution within the clade), and on the lack of any evidence of other genetic systems within the inbreeding Dryocoetini and Xyleborini. The 'undescribed genus' will be described by Bright & Rabaglia (1999). Concerning feeding habits, the outbreeding taxa depicted here feed on phloem of woody plants, with the following exceptions: Micracini feed on xylem, Xyloterini feed on ambrosia (fungi cultured on the walls of tunnels bored into xylem), *Thamnurgus* feed mostly on herbaceous tissues, and *Dactylotrypes longicollis* is unique among outbreeders in being a seed specialist. Among inbreeders, the genera *Coccotrypes*, *Dryocoetiops* and *Hypothenemus* use mostly small plant parts—seeds, fruits, leaf-stalks and the pith of twigs—although *Coccotrypes* also includes phloem feeders. All the 'Xyleborini', including *Premnobius*, are ambrosia feeders, and apparently the undescribed dryocoetine genus feeds on ambrosia as well (Bright & Rabaglia 1999).

ecological niches. The haplodiploid clade has over 1300 species, compared with about 40 in the sister genus (Bright & Skidmore 1997; Wood & Bright 1992), representing a more than 30-fold difference. The lineages traditionally placed in Xyleborini all cultivate fungi ('ambrosia') on the walls of tunnels bored into the xylem, while the inbreeding Dryocoetini feed in phloem or in small, ephemeral resource units like seeds, fruits, mangrove radicles, leaf-stalks and the pith of small twigs. The species that have the ancestral habit (phloem-feeding) are now outnumbered within the haplodiploid clade by seed-feeding species, and especially by the nearly

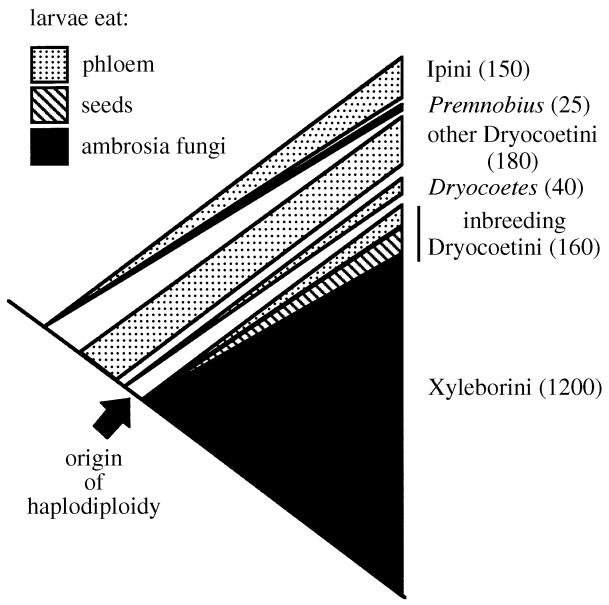

Figure 2. A schematic representation of the diversification of the haplodiploid clade, indicating feeding habits (shading) and species diversity (approximate number of species is given in parentheses and reflected in vertical proportions). For a more specific account of feeding habits, see the caption of figure 1. Only the most thoroughly sampled tribes from figure 1 are included here; the close relationship of Ipini to Dryocoetini + Xyleborini is supported only weakly by the ef-1α and combined analyses presented here, but is corroborated by other molecular evidence, particularly 18S sequences (B. B. Normark, B. H. Jordal and B. D. Farrell, unpublished observations).

1200 species of ambrosia feeders. Our results cannot resolve the sequence of events in the ecological diversification of the haplodiploid clade, which appears to have occurred rapidly, early in the history of the group (B. H. Jordal, B. B. Normark and B. D. Farrell, unpublished data).

The diversification of the haplodiploid clade of scolytids appears to have been fairly recent in geological terms. Bright & Poinar (1994) noted the striking contrast between the great abundance and diversity of the tribe Xyleborini in the neotropics today with its complete absence from Dominican amber (23–40 million years old), which is otherwise rich in ambrosia-feeding beetles (Platypodidae and scolytid tribe Corthylini) and other tribes of scolytids (Scolytini, Ctenophorini, Phloeotribini, Micracini, Dryocoetini and Cryphalini). A relatively recent origin of the haplodiploid clade, compared with the origins of most scolytid tribes, is supported by our ability to resolve its monophyly and sister group here using synonymous substitutions in ef-1α (which has almost no amino-acid replacements in the data presented here).

Scolytids may yet furnish further examples of origins of haplodiploidy, since there remain some sib-mating, female-biased lineages in which the genetic system is completely unknown. The largest such group (with 25 species) is the genus *Premnobius*, which we have discovered to represent a separate origin of sib-mating and of ambrosia feeding. This result was not altogether surprising, as Wood (1986) regarded *Premnobius* as the most distinctive genus in the Xyleborini, and other authors have treated it as a separate tribe, Premnobiini (Browne 1961; Nobuchi 1969). Our finding that *Premnobius* does indeed constitute a separate origin of both inbreeding and ambrosia feeding supports the hypothesis that the association between inbreeding and ambrosia feeding is not a coincidence. Kirkendall (1993) pointed out that larvae that feed by tunnelling through phloem must usually feed alone, while larvae feeding on ambrosia often remain together in a central chamber, facilitating the origin of sib-mating. The only known eusocial beetles, *Platypus incompertus* in the family Platypodidae (Kirkendall *et al*. 1997; Wood 1993), are also ambrosia feeders.

(b) *Evolution of sex-determining mechanisms*

As Bull (1983, p. 172) noted, scolytids may represent the clearest example in nature of Hamilton's favoured scenario for the evolution of haplodiploidy: the habitat (chambers inside wood or other plant tissues) creates situations in which inbreeding occurs, which in turn creates a great selective advantage for female-biased sex ratios, and hence for haplodiploidy as a mechanism that can produce them. But there is another critical factor in Hamilton's and related models of the origin of haplodiploidy: the sex determination mechanism from which haplodiploidy arises (Bull 1983). In Borgia's (1980) analysis, haplodiploidy can arise only from a system with XO male heterogamety or from one with cytoplasmic sex determination. A number of other models of the origin of haplodiploidy also depend on XO males, for instance those in which it evolves to resist (Borgia 1980) or to exploit (Haig 1993) X-chromosome drive. Generally, this prediction is borne out, as most origins of haplodiploidy have been within groups (Acari and Homoptera + Thysanoptera, together accounting for 12 out of the 17 known origins) in which males are otherwise mostly XO (Bull 1983; White 1973). In scolytids, however, (as possibly also in *Micromalthus* and Hymenoptera), haplodiploidy appears to have arisen from a system of XY male heterogamety. The closest relatives of the haplodiploid clade, in the genus *Dryocoetes* (at least *Dryocoetes affaber* and *Dryocoetes autographus*) have XY males, as do most other scolytid species that have been examined (Smith & Virkki 1978). There are two ways to salvage the hypothesis that scolytid haplodiploidy arose from an XO system: (i) one could argue that XO heterogamety arose in the ancestor of the haplodiploid clade, pointing out that it has arisen at least once in Scolytidae (Smith & Virkki 1978); or (ii) one could argue that the peculiar, tiny 'parachute y' sex chromosome of beetles is genetically inert, in spite of some evidence to the contrary (Smith & Virkki 1978). Insights might be gained from studying how brood sex ratios are biased in favour of females in the 'mildly' inbreeding scolytid *Dendroctonus micans*, in which males are not dwarfed or flightless, do occasionally disperse, and bear a diploid chromosome complement including X and Y sex chromosomes (Kirkendall 1993). Some hypotheses also depend on holocentric chromosomes for facilitating the origin of haplodiploidy (Haig 1993; Wrensch *et al*. 1994); again, most origins bear this out (mites, thrips and Homoptera), but scolytids and the other holometabolan origins (*Micromalthus* and Hymenoptera) appear to be exceptions.

More intriguing is the second possible precursor suggested by Borgia for the origin of haplodiploidy—cytoplasmic sex determination. Borgia (1980) cited hints of this in paternal-genome-eliminating diaspidid scale insects, and Hamilton (1979, fig. 6 caption) hinted of it for *Blastophaga* fig wasps, but the clearest evidence actually comes from a haplodiploid scolytid—*Xyleborus ferrugineus*. Peleg & Norris (1972*a*,*b*) and Norris & Chu (1980) have reported that parthenogenetic production of male *X. ferrugineus* is mediated by a transovarially transmitted bacterial endosymbiont. Antibiotic treatment prevented the parthenogenetic production of males temporarily, but in contrast with studies of *Wolbachia*, the 'cure' was not permanent and treated females eventually recovered their ability to produce males. Unfortunately, it was apparently not determined whether treated females could produce daughters if mated. Nonetheless, if Norris and colleagues are correct that a maternally inherited bacterium plays a role in sex determination in haplodiploid scolytids, it may explain why balanced sex ratios and outbreeding have never arisen in any of the over 1300 species in the clade. Sex determination by a maternally inherited element places an extremely low ceiling on the maximum adaptive brood sex ratio (one male per brood) and also favours the reduction of male allocation towards zero under precisely the conditions (presence of non-relatives) that would cause diploid nuclear elements to favour an increase of male allocation towards 50% (Hamilton 1979, fig. 6).

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