



## Evolutionary radiation of an inbreeding haplodiploid beetle lineage (Curculionidae, Scolytinae)

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Haplodiploidy is a highly unusual genetic system that has arisen at least 17 times in animals of varying lifestyles, but most of these haplodiploid lineages remain relatively poorly known. In particular, the ecological and genetic circumstances under which haplodiploidy originates have been difficult to resolve. A recent molecular-phylogenetic study has resolved the phylogenetic position of the haplodiploid clade of scolytine beetles as the sister group of the genus *Dryocoetes*. Haplodiploid bark beetles are remarkable in that the entire clade of over 1300 species are apparently extreme (sib-mating) inbreeders, most of which cultivate fungi for food while some attack phloem, twigs or seeds. Here we present a much more detailed molecular-phylogenetic study of this clade. Using partial sequences of elongation factor 1- $\alpha$  and the mitochondrial small ribosomal subunit (12S), we reconstructed the phylogeny for 48 taxa within the haplodiploid clade, as well as two species of the diploid sister genus *Dryocoetes*. Results indicate that the genus *Ozopemon* is the basal lineage of the haplodiploid clade. Since *Ozopemon*, *Dryocoetes*, and other outgroups are phloem-feeding, this strongly suggests that haplodiploidy and inbreeding evolved in a phloem feeding ancestor. Following the divergence of *Ozopemon* there is a series of extremely short internodes near the base of the clade, suggesting a very rapid rate of diversification in early Miocene (based on fossil evidence and sequence divergence). Among the many substrates for breeding and food resources utilized within this species-rich clade, the cultivation of yeast-like ambrosia fungi in tunnels deep into the wood predominates (nearly 90% of the species). The number of transitions to feeding on such fungi was few, possibly only one, and is perhaps an irreversible transition. The habit of feeding on fungi cultured in xylem makes it possible for the beetles to use a great variety of plant taxa. This extreme resource generalism, in conjunction with the colonization advantage conferred by haplodiploidy and inbreeding, may have promoted the rapid diversification of this clade.

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**ADDITIONAL KEY WORDS:**—ambrosia beetles – bark beetles – Xyleborini – Dryocoetini – fungus feeding – phylogeny – elongation factor 1- $\alpha$  – 12S – amber fossils – diversification.

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## INTRODUCTION

Haplodiploidy, in which diploid females produce haploid males from unfertilized eggs, has arisen at least 17 times in animals that represent a wide variety of lifestyles and habitats (Mable & Otto, 1998). This genetic system results in unusual patterns of relationships within families which have been implicated in the origin of eusociality in Hymenoptera and Thysanoptera (Hamilton, 1964, 1972; Crespi, 1992). Haplodiploidy also makes it possible in principle for a female to precisely control the sex of each offspring, and Hamilton (1967, 1978, 1993) argues that this genetic system is an adaptation for increasing the sex-ratio of taxa that regularly inbreed. Most haplodiploid clades today include many outbreeding species, and reconstruction of the ancestral breeding system is difficult (White, 1973; Nur, 1980; Bull, 1983; Norton *et al.*, 1993; von Dohlen & Moran, 1995; Crespi *et al.*, 1996; Garey *et al.*, 1996). However, there exists one haplodiploid clade—within the weevil subfamily Scolytinae—in which outbreeding is unknown (Kirkendall, 1993; Normark, Jordal & Farrell, 1999).

This haplodiploid clade constitutes at least 70% of the scolytine species in any lowland tropical locale (Beaver, 1979; Beeson, 1961; Browne, 1961), and together with cerambycid larvae, dominates the guild of bark and wood boring beetles throughout the tropics. While the overwhelming majority of these haplodiploid species create galleries within woody hostplant tissues in which they cultivate fungi for both larval and adult food, some also feed on phloem, seeds, or twigs. It has not been clear which of these habits is basal, coincident with the origin of haplodiploidy and inbreeding.

In the entire haplodiploid clade of scolytines (Normark, Jordal & Farrell, 1999)—over 1300 species—matings occur between siblings in the natal nest, or more rarely, between mother and son(s) in a new nest colonized by single females (reviewed in Kirkendall, 1993). This mating strategy is associated with highly female-biased sex ratios and flightless, dwarfed males that have reduced eyes. Extreme inbreeding and highly female-biased sex ratios have also arisen in at least six other lineages of scolytines (Kirkendall, 1993; Normark, Jordal & Farrell, 1999), one of which is

known to be pseudoarrhenotokous—having functionally haploid males arising from fertilized eggs (Brun *et al.*, 1995)—further enhancing the potential value of scolytines for understanding the origin and adaptive significance of haplodiploidy.

There have only been two radiations of haplodiploid lineages in holometabolous insects, one comprising the haplodiploid clade of scolytines and the other the entire order Hymenoptera (Mable & Otto, 1998). A third known origin of haplodiploidy in Holometabola, in the bizarre beetle family Micromalthidae, seems to have been a dead end, as there is only one species known. An understanding of the ecological circumstances of the origin of haplodiploidy in Hymenoptera has been much sought after, but has remained elusive, mostly because the phylogenetic affinities of Hymenoptera have remained uncertain (Vilhelmsen, 1997). Recently, for the haplodiploid clade of scolytines, we have unambiguously identified its sister-group as the outcrossing, phloem-feeding genus *Dryocoetes*, but relationships *within* the haplodiploid clade have remained obscure (Normark, Jordal & Farrell, 1999).

An understanding of phylogenetic relationships within the haplodiploid clade of scolytines is critical to understanding both the causes of the transition to haplodiploidy at the origin of the group and the possible consequences for subsequent evolutionary change in feeding habits and diversity. The range of feeding modes within the haplodiploid clade is extremely wide; hence different hypotheses about the group's phylogeny imply very different scenarios of the circumstances (and possible causes) of the transition to haplodiploidy, and also imply very different sequences of subsequent ecological transitions. Indeed, the various lineages of the haplodiploid clade are so diverse in their habits that they have traditionally been sorted into two different tribes (Wood, 1986), with seed-feeding and phloem-feeding species (bark beetles, in the ecological sense) in the tribe Dryocoetini and the almost nine-fold greater number of fungus-cultivating species (ambrosia beetles) in the tribe Xyleborini.

In order to help clarify the sequence of events surrounding the origin of haplodiploidy and extreme inbreeding and their possible relationship to fungus cultivation in these scolytine beetles, we have conducted an intensive molecular-phylogenetic study of relationships within the haplodiploid clade, using partial sequences of the nuclear gene elongation factor 1-alpha (ef-1 $\alpha$ ) and the mitochondrial small subunit ribosomal gene (12S). Building on our previous study of the phylogenetic position of the haplodiploid clade within the subfamily Scolytinae (Normark, Jordal & Farrell, 1999), this study succeeds in resolving some critical relationships within the haplodiploid clade, where that study failed. The new resolution obtained by this study has been possible because we have used a new locus (12s) and because we have now managed to sample many additional taxa. We have more than doubled the number of haplodiploid species sampled, and added several additional genera—including, most crucially, the rare Southeast Asian phloem-feeding genus *Ozopemon*, which has bizarre larviform males (Browne, 1959; Jordal *et al.*, in prep.).

## MATERIAL AND METHODS

### *Genes and taxa*

We collected and analysed 873 base pairs from the 5' end of ef-1 $\alpha$  and 326 base pairs from the 5' end of 12S. The choice of *Dryocoetes* as the outgroup was based on

its unambiguous placement as the sister group to the haplodiploid clade as shown previously (Normark, Jordal & Farrell, 1999). The species analysed, their geographical sources, and their main food resources are listed in the Appendix; their GenBank accession numbers are AF186659–AF186661, AF186664, AF186668–AF186670, AF186684–AF186688, AF186690–AF186696, AF259863–AF259893 (ef-1 $\alpha$ ) and AF259813–AF259862 (12S). Most samples were collected into 100% ethanol; a few were frozen in liquid nitrogen, stored in 70% ethanol, or dried. Voucher specimens are deposited at the Museum of comparative Zoology, Harvard University, or in Department of Zoology, University of Bergen.

There are two copies of ef-1 $\alpha$  in scolytine beetles, distinguished both by the positions of introns and by amino acid sequence; here, as previously (Normark, Jordal & Farrell, 1999), we use only one of the two putative loci—the one that has a single intron, in the same position as the second intron in the 'F2' sequence of *Apis* (Danforth & Ji, 1998). The two copies are unlikely to be confused, in part because of the several amino acid replacement substitutions that consistently distinguish them. We obtained the second (two-intron) copy for one of the species considered here (*Coccotrypes advena*), and the degree of divergence in implied amino acid sequence between the two copies in this species (6.2%) is well outside the range of divergences for the one-intron copy across the haplodiploid clade (0–2.5%).

#### *DNA extraction, PCR and sequencing*

Whole individuals (adults, pupae or larvae) or just thorax and head (for some large specimens) were ground in liquid nitrogen, and DNA was extracted using either a 'salting out' protocol (Sunnucks & Hales, 1996) or the Qiagen QIAamp Tissue Kit. For ef-1 $\alpha$ , PCR protocols and list of primers are given in Normark, Jordal & Farrell (1994). For 12S, we used the primers SR-J-14233 and SR-N-14588 of Simon *et al.* (1994), and 40 cycles of the following temperature profile: 95°C denaturing for 30 s, 47°C annealing for 60 s, and 72°C extension for 60 s. 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.

#### *Sequence editing and alignment*

The sequences were compiled and edited for base call errors using Sequencher 3.0 (Gene Codes Corporation, Ann Arbor, Michigan), and in nearly all cases were checked by reading both strands. Only unambiguous base calls were included from the few regions that were read in only one direction. For ef-1 $\alpha$ , the sequence of the intron was omitted from the phylogenetic analysis. There were no insertions or deletions in the protein-coding sequence, so alignment was unambiguous. Alignment of 12S was done in ClustalX (Thompson *et al.*, 1997) using the gap:extension:change cost of 15:15:1 and thereafter adjusted by eye. There was little variation in alignments under different weighting regimes, because few insertions or deletions were necessary. Eight sites were omitted due to ambiguous alignment.

*Phylogenetic analysis*

As a guide to identify the level of saturation due to multiple substitutions, we used MEGA 1.02 (Kumar, Tamura & Nei, 1993) to plot the number of transition differences versus the number of transversion differences for all pairwise sequence comparisons for each gene separately.

Paup\* 4.0 (Swofford, 1999) was used for all phylogenetic analyses. We combined the ef-1 $\alpha$  and 12S matrices for phylogenetic analyses, regardless of their level of congruence, a procedure that maximizes explanatory power (Kluge, 1989; Kluge & Wolf, 1993) and the ability to detect phylogenetic signal (Baker & DeSalle, 1997; Remsen & DeSalle, 1998). However, we also analysed each gene separately, and used the saturation plots, in conjunction with overall bootstrap support, to examine the relative contribution of signal to noise in each data set.

Under the maximum parsimony (MP) criterion we performed 100 random addition replicates of heuristic searches; bootstrap support for individual nodes were assessed by 100 bootstrap replicates (Felsenstein, 1985) of 10 random addition heuristic searches each. A maximum likelihood model of nucleotide evolution for minimum-evolution (ME) distance analyses was used as a substitute for a full maximum likelihood analysis due to the high number of terminal taxa. Starting parameters were estimated as an average from the unweighted MP trees and from a ME tree with Kimura 2-parameter corrected distances. Parameters refined by iterative searches were used in the final analysis of 100 random addition replicates of heuristic searches.

To test whether certain topologies, expected from morphological data, were significantly longer than the most parsimonious topology from the unweighted combined data set, we repeated the searches with the following topological constraints: inbreeding Dryocoetini monophyletic and Xyleborini basal; Xyleborini monophyletic and inbreeding Dryocoetini basal; each of the inbreeding Dryocoetini and Xyleborini monophyletic; *Coccotrypes* monophyletic; *Xyleborus* monophyletic. Using Templeton's Wilcoxon signed rank test (Templeton, 1983), and Kishino & Hasegawa (1989) test, as implemented in PAUP\*, we compared the globally shortest trees to the shortest trees under each constraint.

## RESULTS

The level of saturation due to multiple substitutions was low for ef-1 $\alpha$ , and moderate for 12S (Fig. 1), a pattern supported by the high (ef-1 $\alpha$ ) and moderate (12S) average ratio of transitional to transversional substitutions for the two genes (Table 1).

All combined analyses supported a sister-group relationship between *Ozopemon* and all remaining inbreeding taxa (Figs 2, 3), and separate analyses of ef-1 $\alpha$  and 12S did not contradict this result. The ef-1 $\alpha$  strict consensus tree (from 365 MP trees) contained only nodes also present in the combined analysis. Although the separate analysis of 12S did not provide much resolution beyond intrageneric level, it did contribute to elevate bootstrap support in the combined analysis, for 16 out of the 21 comparable (resolved) nodes from the ef-1 $\alpha$  consensus tree. The latter result illustrates how combined analysis can amplify weak phylogenetic signal from

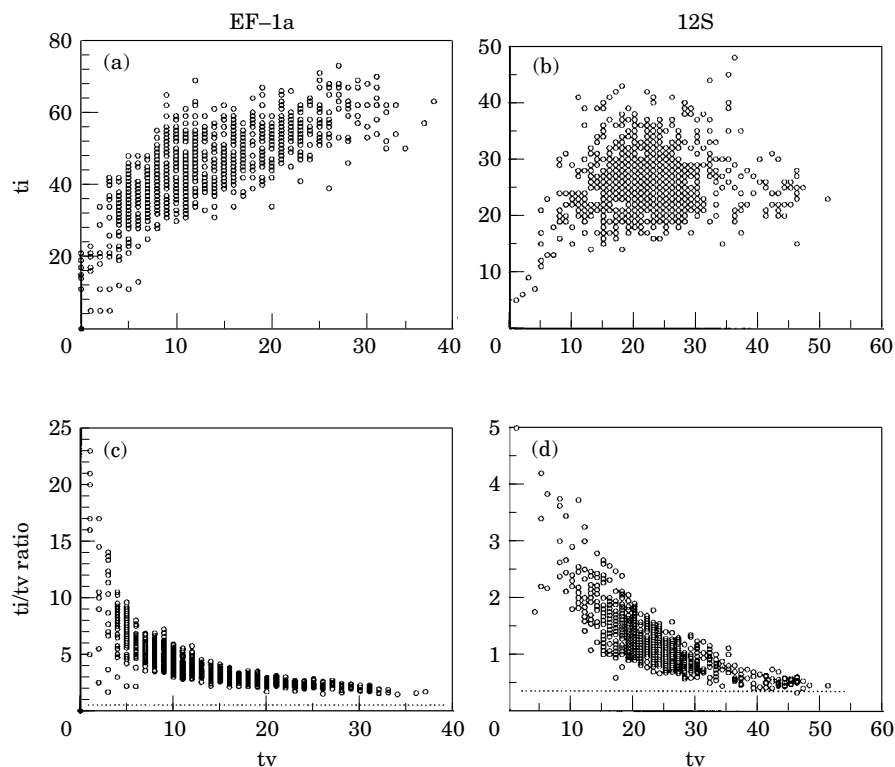


Figure 1. Plots of transitions (A, B) and transition/transversion ratios (C, D) against transversions for all pairwise sequence comparisons. None of the comparisons for ef-1 $\alpha$  (C) and rather few for 12S (D) were completely saturated, as indicated by the expected ti/tv ratio (Holmquist, 1983) shown by the stippled lines (0.55 and 0.38). Hence, we did not exclude any character partitions for the phylogeny analyses.

TABLE 1. Properties of gene subsets. Numbers given in parentheses are based on the ingroup only. Heterogeneity of base frequencies across taxa was not significant for either genes or for any of the ef-1 $\alpha$  positions. Pairwise sequence divergences are uncorrected; Ti and Tv signify transitions and transversions, respectively

Gene subset	bp	Informative sites	Mean sequence divergence	Max sequence divergence	Ti/Tv				
					(mean)	% A	% C	% G	% T
12s, all pos.	326	140 (140)	15.3 (15.3)	23.1 (23.1)	1.19	38.9	18.2	8.8	34.1
EF-1 $\alpha$ , all pos.	873	238 (230)	7.0 (6.8)	12.1 (11.7)	3.34	27.1	22.3	23.9	26.7
EF-1 $\alpha$ , 1 pos.	291	19 (18)	1.3 (1.3)	3.6 (3.2)	2.51	28.8	17.0	38.9	15.3
EF-1 $\alpha$ , 2 pos.	291	6 (4)	0.4 (0.3)	2.5 (1.4)	0.49	30.8	24.7	16.0	28.5
EF-1 $\alpha$ , 3 pos.	291	213 (208)	19.4 (18.8)	32.8 (32.8)	3.58	21.6	25.3	16.9	36.2

separate analyses to a perceptible signal in the combined analysis (see Baker & DeSalle, 1997 for a discussion on this matter), and justifies combined analysis.

Most intergeneric relationships in the combined analysis were weakly resolved, with low bootstrap support. In contrast, five out of eight intrageneric relationships were strongly supported. *Xylebours* is polyphyletic, while *Coccotrypes* is paraphyletic

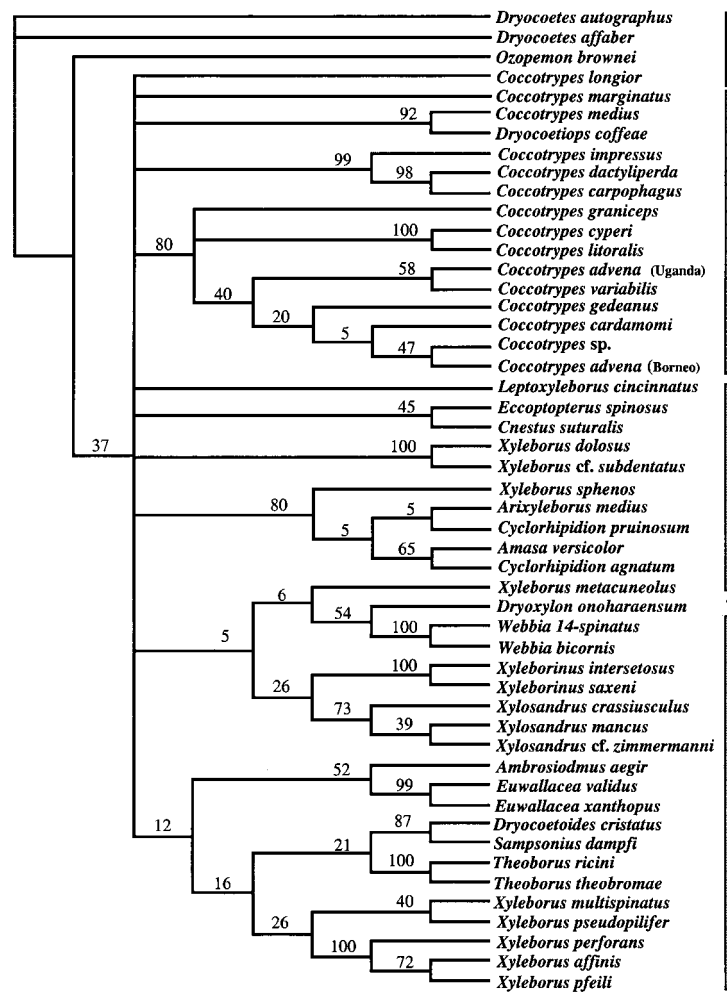


Figure 2. Strict consensus tree of the two most parsimonious trees from the parsimony analysis. Treelength = 2430 steps, CI (informative characters) = 0.27, RI = 0.40. Numbers above nodes are bootstrap support values. In the right margin, open bar (fungus feeding) indicates Xyleborini, and solid (phloem feeding) and obliquely hatched (seed, twig, petiole, and phloem-feeding) bars indicate Dryocoetini as currently classified (Wood & Bright, 1992). *Dryoxylon* is tentatively placed in Dryocoetini by Bright & Rabaglia (1999), but is wood-boring and probably fungus-feeding, a trait not found in other dryocoetines.

with respect to *Dryocoetiops*. The 'dolosus' group of *Xyleborus* also appeared in the *Coccotrypes* clade in the ME analysis, though only with low bootstrap support. None of these poly- or paraphyletic groups were monophyletic in either of the separate analyses. It should be noted that the genera *Xyleborus* (565 spp.) and *Coccotrypes* (119 spp.) are currently in a state of taxonomic chaos, monophyly is not expected, and reclassification is much needed (Wood, 1986).

Of particular interest is the monophyly, in most analyses, of a group consisting of three genera endemic to the Neotropics (*Theoborus*, *Sampsonius*, and *Dryocoetoides*; e.g. Wood, 1986). The clade consisting of the nest parasite genus *Sampsonius* and its

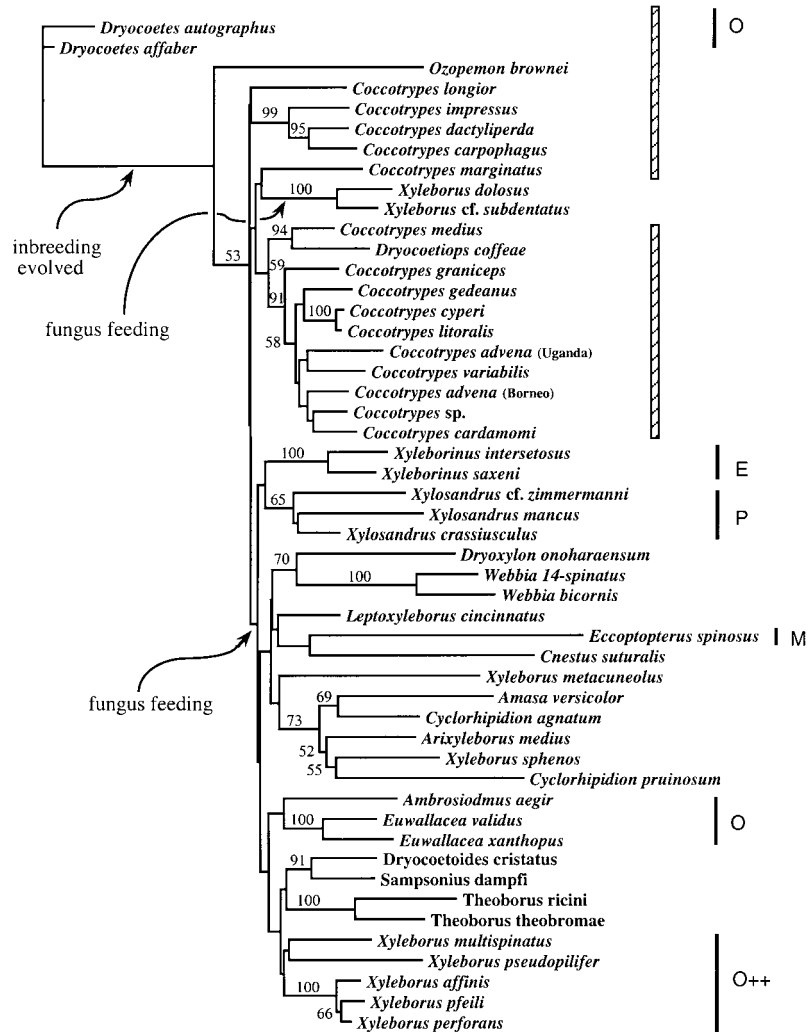


Figure 3. The single tree that resulted from the ME analysis with ML distances. The proportion of invariant sites was estimated to be 0.50 and the shape parameter of the gamma distribution 0.60. Bootstrap support values higher than 50% are indicated on nodes. The monophyletic clade of three Neotropical endemic genera is indicated by plain text taxon names, the two origins of fungus-feeding by arrows, the phloem/seed feeding taxa by obliquely hatched bars, and capital letters in conjunction with solid bars indicate position of mycangia (paired, spore-bearing cavities in the cuticle) when known from one or more species of a genus. Abbreviations: E = base of elytra; P = posterior pronotum; M = mesonotum; O = oral pouches; ++ = many different types.

common host genus *Dryocoetoides* (see Wood, 1982) was one of very few highly supported intergeneric groups (Figs 2, 3).

Constrained searches for alternative topologies increased the tree lengths marginally, from 0.2% to 0.9% compared to the globally most parsimonious trees. Only the monophyly of *Xyleborus* and of *Coccotrypes* could be rejected in one of the tests (Kishino-Hasegawa—ML criterion,  $P=0.01, 0.04$ ). Neither the monophyly of inbreeding Dryocoetini nor Xyleborini could be rejected ( $P$ -values  $>0.26$  for all tests).

## DISCUSSION

*Phloem, cradle of inbreeding and haplodiploidy*

Our results support the hypothesis proposed by Browne (1959) and Nobuchi (1969), based on adult morphology, and Lekander (1968) and Gardner, (1934), based on larval characters, that *Ozopemon* represents a transition from outbreeding Dryocoetini to the inbreeding taxa, and that *Coccotrypes* is more closely related to Xyleborini. Although weakly supported by bootstrap values, the basal placement of *Ozopemon* was consistent in all analyses. *Ozopemon* shares its habits of exclusive phloem feeding with *Dryocoetes*, the closest outgroup to the haplodiploid clade (Fig. 3), as well as almost all other outbreeding Dryocoetini (Normark, Jordal & Farrell, 1999). Also, the relatively basal placement (Fig. 3) of *C. longior* (Eggers), one of rather few phloem feeding species in *Coccotrypes*, is consistent with the hypothesis that inbreeding and haplodiploidy originated within a phloem-feeding lineage.

The basal placement of the gregariously phloem feeding *Ozopemon* also suggests that inbreeding can evolve in phloem without specific spatial constraints as those imposed by breeding in small and ephemeral resource units (e.g. seeds, leafstalks, and twigs), or in association with fungal colonies. Thus, there is no reason to believe that such unconventional breeding and feeding substrates are necessary requirements for inbreeding (and subsequently, haplodiploidy) to evolve, as recently argued (Kirkendall, 1993).

*Radiation*

An exceptionally wide array of different feeding modes and behaviours evolved subsequent to the origin of haplodiploidy; thus it is tempting to suppose that this may have been triggered by this peculiar genetic system. Indeed, the haplodiploid lineage appears to be the most species-rich clade of comparable age in Scolytinae and has more than 30 times as many species as its putative sister group *Dryocoetes* (c. 40 spp). While several model-based tests of diversification have been proposed that would likely underscore the asymmetry of this sister group diversity contrast (Sanderson & Donoghue, 1996; Slowinsky & Guyer, 1993), evidence in addition to pronounced tree asymmetry seems necessary to infer process (Farrell, Dussourd & Mitter, 1991). Moreover, variation in feeding modes within this clade raises the possibility that changes in resource use (in particular, cultivating fungi within hostplants) have also been important in diversification (Farrell, 1998).

The most species rich group in the haplodiploid clade is the fungus cultivating Xyleborini, which thus bears most of the diversity that might be explained. The analysis that yielded the greatest resolution (Fig. 3) shows two origins of fungus feeding, with no reversals. The two trees resulting from unweighted parsimony analysis (not shown) also indicate two origins, but with two reversals. Traditional classification implies a single origin (if Xyleborini is monophyletic), and we cannot reject this hypothesis. While eventual incorporation of morphological characters in the matrix may yet indicate a single origin, additional, indirect evidence favours multiple origins and subsequent irreversibility of fungus-feeding.

First, many of those fungi found in association with phloem-feeding species are also the most important components in the complex of fungi and bacteria upon

which ambrosia beetles are obligately dependent (Beaver, 1989; Guadalupe Rojas, Morales-Ramos & Harrington, 1999; Six & Paine, 1998). Taken together with the convergent evolution of spore-bearing mycangia (paired cuticular cavities) in the at least 11 independent origins of fungus feeding in the Scolytinae (not counting Platypodidae), and the presence of similar mycangia even in some fungus associated, but phloem-feeding species (table II in Beaver, 1989), the transition to obligate fungus feeding seems a relatively simple step.

Moreover, parallel evolution of the remarkable array of non-homologous mycangia across different genera within the Xyleborini (Fig. 3) strongly suggests adaptation and underscores the importance of effective vectoring of these microbes to hostplant tissues. Beetle lineages in which this intimate association with ambrosia fungi is established may be permanently dependent on the fungi and unable to revert to direct feeding on woody tissues. First, the anterior (grinding) plate of the proventriculus, apparently necessary to prepare tough tissues (Nobuchi, 1969, 1972), is absent in all ambrosia feeding beetles. Once beetles are adapted to feed exclusively on fungi, there is a pronounced reduction in the grinding capability of the proventriculus. Second, at least some *Xyleborus* are dependent on the fungal constituent ergosterol for successful oocyte maturation, egg hatching and pupation (Kok, Norris & Chu, 1970; Kukor & Martin, 1987), a dependence that might be widespread in the Xyleborini. Fungus feeding may thus be irreversible. This highly specialized feeding mode, which is accompanied by marked increase in the breadth of hostplant species used by single beetle species (Beaver, 1979), would seem to ensure frequent contact of close relatives, fostering opportunities for competition and other antagonistic interactions.

It may not be surprising then that the ambrosia-feeding clade has given rise to the inquiline nest parasite behaviour of the genus *Sampsonius* (see Wood, 1982). Species of this genus are incapable of excavating an entrance tunnel and take over an existing tunnel system made by their host, another xyleborine ambrosia beetle. The close relationship found here, between *Sampsonius* and their most common host genus *Dryocoetoides* (Figs 2, 3), provides support for a relatively little tested evolutionary pattern, known as Emery's rule, whereby parasites are closely related to their hosts (Wilson, 1971). A similar strategy is found among a few other Neotropical scolytine species, in the outbreeding ambrosia beetles *Gnathotrupes* (see Naumann-Etienne, 1978) and *Tricolus* (see Wood, 1982) which use conspecifics as hosts.

#### *Rapid diversification?*

Lack of resolution at deeper nodes is striking in this study (Fig. 3). Some recent studies suggest that this lack of resolution is an expected consequence of especially rapid adaptive radiation (as opposed to idiosyncrasies of the molecular markers surveyed), where lineages diversify following a key innovation without production of many apomorphies (e.g. Garcia-Moreno, Arctander & Fjelds , 1999; Hodges, 1997; Jackman *et al.*, 1999; Lovette & Bermingham, 1999; von Dohlen & Moran, 2000). Because ML branch lengths are a function of both time and substitution rate, short nodes could either indicate rapid speciation or a drastic slowdown in rate (e.g. Hodges, 1997). However, though fossil dated nodes are needed to distinguish between the two, a slowdown in rate is the least probable outcome of speciation events (Avice, 1994). Furthermore, the short internodes at the base of the tree (Fig.

3) are unlikely to be an artefact of insufficient sampling of characters, since the number of informative characters (Table 1) amounts to eight times the number of taxa. Nor are they likely to be due to saturation, because the level of substitutional saturation is low, especially for  $ef-1\alpha$  (Fig. 1;  $ef-1\alpha$  produced equally short internodes in the separate analysis).

While we must remain circumspect in interpreting lack of resolution in particular studies, similar patterns of rapid diversification have also been suggested for the lack of resolution observed between tribes of aphids which diversified rapidly in association with angiosperms (von Dohlen & Moran, 2000) as well as for the major mammalian radiation in early Tertiary (Springer, 1997). In fact, it is expected that rapidly expanding groups will tend to have star-like tree topologies with very short internal branches separating clades (Otto, Cummings & Wakely, 1996; Slatkin & Hudson, 1991). That empirical results have been robust to tests of the reality of rapid diversification (that is, hard *versus* soft polytomies), as for *Anolis* lizards (Jackman *et al.*, 1999), and auklets (Alcidae) (Walsh *et al.*, 1999), further strengthen the view that starburst phylogenies may result from rapid diversification. While we tentatively suggest that the short internodes observed deep in our topology (Fig. 3) may be the result of rapid diversification, it remains to be demonstrated which combination of features—close inbreeding and haplodiploidy, or fungus feeding—are responsible for the diversity increase. Comparative study of other instances of each of these traits, presently underway, will help resolve their possible influence on diversification.

#### *Timing of the radiation: fossil evidence*

A comparatively rich fossil record of bark and timber beetles from Baltic amber (28–50 Mya) and Dominican amber (23–40 Mya) provides a promising lead to the age of the haplodiploid lineage. The complete absence of any members of the haplodiploid clade from both types of amber (Bright & Poinar, 1994; Larsson, 1978) would seem to suggest an origin no earlier than the mid-Tertiary. However, Baltic amber, of coniferous origin, contains mainly temperate elements of today's faunas (Larsson, 1978; Wood, 1982) and it is possible that the absence of species related to the haplodiploid clade is due to unfavourable climate. Thus, particularly illuminating is the angiosperm-originating Dominican amber, since it represents a tropical fauna from a region where Xyleborini are currently prominent in the ambrosia-feeding guild, together with Corthyliina and Platypodidae (Noguera-Martinez & Atkinson, 1990). All three groups are attracted to resinous fumes (terpenes) (Barbosa & Wagner, 1988), and xyleborine beetles as well as corthyliines and platypodids are among the first beetles expected to be trapped in resin exudates (pers. obs., BHJ). Therefore the observation that platypodids and corthyline ambrosia beetles are abundant in Dominican amber, while Xyleborini are absent (Bright & Poinar, 1994), strongly suggests that Xyleborini were not present in the New World 23 Mya, but instead radiated rapidly in the Miocene.

The possibility remains, though, that the haplodiploid clade originated in the Old World prior to the New World radiation. Indeed, the most basal taxon, *Ozopemon*, is an endemic Asian genus, and 20 out of the 24 remaining genera are known from that region (Wood & Bright, 1992), suggesting the area of origin. An early Oligocene rather than Miocene origin also seems likely given the high level of sequence divergence within this clade—the average divergence in  $ef-1\alpha$  third positions (18.8%,

Table 1) equals that of 'trifine' Noctuidae, a radiation some 30–40 Mya old (Mitchell *et al.*, 1997). An early Oligocene age is further supported by the presence of taxa closely related to the haplodiploid clade in the 45 Mya old Baltic amber, namely *Taphramites* (related to *Thamnurgus*) and species of the extant genus *Taphrorychus* (Larsson, 1978; Normark, Jordal & Farrell, 1999). However, the presence of outbreeding dryocoetines in the younger Dominican amber (i.e. *Dryomites* (Bright & Poinar, 1994), underscores the importance of the absence of xyleborines in these Dominican deposits.

Together, this combination of molecular and fossil evidence indicates existence of the ancestral outbreeding lineage in the Eocene, and a very rapid radiation no earlier than the early Oligocene or in the Miocene. The production of nearly 1400 species in such a brief span of time seems a classic adaptive radiation.

#### *The interplay of resource use and mating system*

While we cannot yet make strong inferences concerning the source of the extraordinary diversity of these beetles, we hypothesize that the synergy of two features might explain their extraordinary success. First, these beetles are mainly extreme substrate generalists, which is particularly advantageous in heterogeneous tropical forests (Beaver, 1979). For instance, while many species of *Coccotrypes* use a broad range of plant tissues, including seeds, leaves and phloem, the Xyleborini are able to use of a great variety of host-plant taxa. This is possible because these ambrosia beetles specialize instead on the apparently mutualistic fungi themselves, which are the actual hostplant generalists (Browne, 1958; Beaver, 1989; Norris, 1992). The fungal association has thus made it possible for ambrosia beetles to utilize a higher proportion of available hostplants than host-tissue feeding bark beetles are able to (Beaver, 1979; Atkinson & Equihua-Martinez, 1986) and hence to become among the most extreme host-plant generalists in the tropics.

Such broad resource use arose in these beetles following the origins of haplodiploidy and regular sib-mating, a concordance of life-history traits that should enhance their rate of diversification. Coupled with haplodiploidy, sib-mating conveys an advantage in the establishment of new populations in isolated areas, since it frees females of the necessity to find mates outside her natal nest. Because even a virgin female can found a new population by mating with parthenogenetically produced sons, it may not be coincidental that small tropical islands have a significantly higher portion of inbreeding scolytine species than have the mainland or larger islands (Kirkendall, 1993). Indeed, higher than average potential of inbreeding scolytines for successful dispersal may also be reflected in their dominance in recent introductions, for example to North America (Wood, 1982; Atkinson, Rabaglia & Bright, 1990). The combination of inbreeding and haplodiploidy provides an additional advantage in that it enables the consistent production of extremely female-biased sex ratios, which are expected to nearly double the reproductive rate (Hamilton, 1967). The combination of a broadened base of available resources (due to ambrosia-feeding) and an enhanced ability to propagate (due to haplodiploidy and sib-mating) may spur the radiation of these beetles. Tests of this hypothesis are facilitated by the independent origins of ambrosia-feeding and inbreeding in other groups of bark beetles, providing opportunities for comparative study of the effect of each of these

characters on abundance, geographic range, modes of speciation, and other potential components of diversification.

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## APPENDIX

Collecting area for the samples sequenced in this study along with their main food resource(s). Alias refers to voucher specimens in BDF laboratory, MCZ.

Tribe	Species	Alias	Collected	Food source	
Dryocoetini	<i>Coccotrypes advena</i>	scd24	Uganda	seed, fruit, petiole	
	<i>Coccotrypes advena</i>	scd36	Borneo	seed, fruit, petiole	
	<i>Coccotrypes cardamomi</i>	scd38	Japan	seed, fruit, petiole, fern, phloem	
	<i>Coccotrypes carpophagus</i>	scd43	USA	seed	
	<i>Coccotrypes cyperi</i>	scd11	Costa Rica	seed, fruit, petiole, phloem	
	<i>Coccotrypes dactylipenda</i>	scd14	Argentina	seed	
	<i>Coccotrypes gedeanus</i>	scd35	Borneo	seed, fruit, petiole	
	<i>Coccotrypes graniceps</i>	scd31	Japan	seed	
	<i>Coccotrypes impressus</i>	scd54	Thailand	seed	
	<i>Coccotrypes litoralis</i>	scd12	Bangladesh	mangrove radicle or fruit	
	<i>Coccotrypes longior</i>	scd40	Borneo	phloem, petiole	
	<i>Coccotrypes marginatus</i>	scd34	Singapore	petiole	
	<i>Coccotrypes medius</i>	scd55	Singapore	phloem, petiole, fruit	
	<i>Coccotrypes</i> sp.	scd32	Japan	seed, ?other	
	<i>Coccotrypes variabilis</i>	scd30	Japan	fruit, seed, petiole, phloem	
	<i>Dryocoetes affaber</i>	scd20	USA	phloem	
	<i>Dryocoetes autographus</i>	scd53	Japan	phloem	
	<i>Dryocoetiops coffeae</i>	scd37	Borneo	pith	
	<i>Dryoxylon onoharaensum</i>	scd19	USA	?fungus	
	<i>Ozopemon brownei</i>	scd39	Borneo	phloem	
	Xyleborini	<i>Amasa versicolor</i>	scy40	Borneo	fungus
		<i>Ambrosiodmus aegir</i>	scy19	Uganda	fungus
		<i>Arixyleborus medius</i>	scy37	Borneo	fungus
<i>Cnestus suturalis</i>		scy36	Borneo	fungus	
<i>Cyclorhipidion agnatum</i>		scy51	Borneo	fungus	
<i>Cyclorhipidion brunosum</i>		scy38	Borneo	fungus	
<i>Dryocoetoides cristatus</i>		scy12	Uganda	fungus	
<i>Eccoptopterus spinosus</i>		scy11	Uganda	fungus	
<i>Euwallacea validus</i>		scy23	USA	fungus	
<i>Euwallacea xanthopus</i>		scy52	Uganda	fungus	
<i>Leptoxyleborus cincinnatus</i>		scy45	Borneo	fungus	
<i>Sampsonius dampfi</i>		scy41	Brazil	fungus	
<i>Theoborus ricini</i>		scy18	Uganda	fungus	
<i>Theoborus theobromae</i>		scy33	Costa Rica	fungus	
<i>Webbia 14-spinatus</i>		scy35	Borneo	fungus	
<i>Webbia bicornis</i>		scy39	Borneo	fungus	
<i>Xyleborinus intersetosus</i>		scy03	Costa Rica	fungus	
<i>Xyleborinus saxeni</i>		scy10	USA	fungus	
<i>Xyleborus</i> cf. <i>subdentatus</i>		scy48	Borneo	fungus	
<i>Xyleborus affinis</i>		scy13	Uganda	fungus	
<i>Xyleborus dolosus</i>		scy46	Borneo	fungus	
<i>Xyleborus metacuneolus</i>		scy50	Singapore	fungus	
<i>Xyleborus multispinatus</i>		scy17	Uganda	fungus	
<i>Xyleborus perforans</i>		scy30	Japan	fungus	
<i>Xyleborus Pfeili</i>		scy29	Japan	fungus	
<i>Xyleborus pseudopilifer</i>		scy47	Borneo	fungus	
<i>Xyleborus sphenos</i>		scy20	Uganda	fungus	
<i>Xylosandrus crassiusculus</i>		scy49	Borneo	fungus	
<i>Xylosandrus mancus</i>		scy34	Singapore	fungus	
<i>Xylosandrus</i> cf. <i>zimmermanni</i>		scy05	Argentina	fungus	