Recent Intron Gain in Elongation Factor-1α of Colletid Bees (Hymenoptera: Colletidae)

Seán G. Brady[1] *and Bryan N. Danforth*

Department of Entomology, Cornell University, Ithaca, New York

We discovered the presence of a unique spliceosomal intron in the F1 copy of elongation factor-1α (EF-1α) restricted to the bee family Colletidae (Hymenoptera: Apoidae). The intron ranges in size from 101 to 1,044 bp and shows no positional sliding. Our data also demonstrate the complete absence of this intron from exemplars representing all other bee families, as well as from close hymenopteran relatives. A review of the literature finds that this intron is likewise absent from all other arthropods for which data are available. This provides unambiguous evidence for a relatively recent intron insertion event in the colletid common ancestor and, at least in this specific instance, lends support to the introns-late hypothesis. The comparative distribution of this novel intron also supports the monophyly of Colletidae and the exclusion of the Stenotritidae from this family, providing an example of the potential of some introns to act as robust markers of shared descent.

Introduction

Explaining the distribution of introns in eukaryotic protein-coding genes is one of the major challenges in contemporary evolutionary genomics. Although some progress has been achieved toward understanding the dynamics of intron evolution, refining this work will depend on expanding our knowledge of the comparative distribution of these macromutations among eukaryotic organisms (Lynch and Richardson 2002).

One trend already evident is that some spliceosomal introns have originated relatively recently in the evolutionary history of eukaryotes (Hankeln et al. 1997; Logsdon 1998; Tarrío, Rodríguez-Trelles, and Ayala 1998). Such cases of recent intron gain have been used to bolster the "introns-late" theory, which maintains that spliceosomal introns are introduced into previously continuous protein-coding regions (Rogers 1990; Cavalier-Smith 1991; Palmer and Logsdon 1991). Although proponents of the alternative "introns-early" theory (Doolittle 1978; Gilbert 1979) contend that deletion of introns ancestral to all eukaryotes is mainly responsible for contemporary intron distributions among taxa, they also recognize that intron insertion occurs to some degree (de Souza et al. 1998; Fedorov, Merican, and Gilbert 2002). Well-documented specific cases of recent intron gain, however, are few in the literature.

In this paper we report the discovery of a novel intron in the F1 copy of elongation factor 1α (EF-1α). This nuclear gene encodes a protein involved in the GTP-dependent binding of charged tRNAs to the acceptor site of the ribosome during translation (Maroni 1993). EF-1α is known to possess two paralogous copies in bees (Danforth and Ji 1998), other Hymenoptera (present study), beetles (Jordal 2002), and flies (Hovemann et al. 1988), whereas only a single copy has been reported from other arthropod taxa.

Materials and Methods

We extracted DNA from individual specimens using a standard phenol-chloroform protocol detailed in Danforth (1999). We generated double-stranded PCR products using Promega (Madison, Wis.) Taq DNA polymerase. For most taxa, we amplified the F1 copy of EF-1α with the following amplification primer pair: For1deg (forward) 5′-GY ATC GAC AAR CGT ACS ATY G-3′ (Danforth, Conway, and Ji 2003) and Rev2 (reverse) 5′-YTC SAC YTT CCA TCC CTT GTA CC-3′ (current study) (fig. 1). In a few cases where amplification product was weak or absent, we used the alternative forward primer For2 5′-AAG GAG GCS CAG GAG ATG GG-3′ (current study). PCR amplifications were conducted using the following cycle conditions: 94°C, 1 min denaturation; 55°C, 1 min annealing; and 72°C, 1 to 2 min extension. The longer extension times were used in some cases with large intron insertions. A concentration of 2.5 mM of $MgCl_2$ was used in a final reaction volume of 75 μl.

All PCR products were gel-purified in low-melting-point agarose (BMA, Rockland, Me.) gels overnight at 4°C. DNA was recovered from gel slices using the Promega Wizard PCR Preps DNA Purification kit. Automated sequencing of both strands was accomplished using the same amplification primers through the Cornell Automated Sequencing Facility. In three taxa, readable sequences were not obtained from the internal portion of the intron because of indel variation. However, in these cases, our sequences spanned both the upstream and downstream intron/exon boundaries, and extended far enough into the intron (usually several hundred base pairs on each side) to unambiguously determine these as intron sequences. Complete intron sequences were obtained in all other cases. Sequence chromatograms were assembled and edited using Sequencher version 4.1 (GeneCodes Corporation, Ann Arbor, Mich.). Sequence alignments were generated with the Lasergene DNA Star software package using ClustalW and improved by eye. Intron/exon boundaries and reading frames were determined by comparison with published sequences for the honeybee, *Apis mellifera* (Walldorf and Hovemann 1990).

[1] Present address: Laboratory of Analytical Biology and Department of Entomology, Smithsonian Institution, MRC 534, Suitland, Maryland 20746.

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E-mail: brady.sean@nmnh.si.edu.

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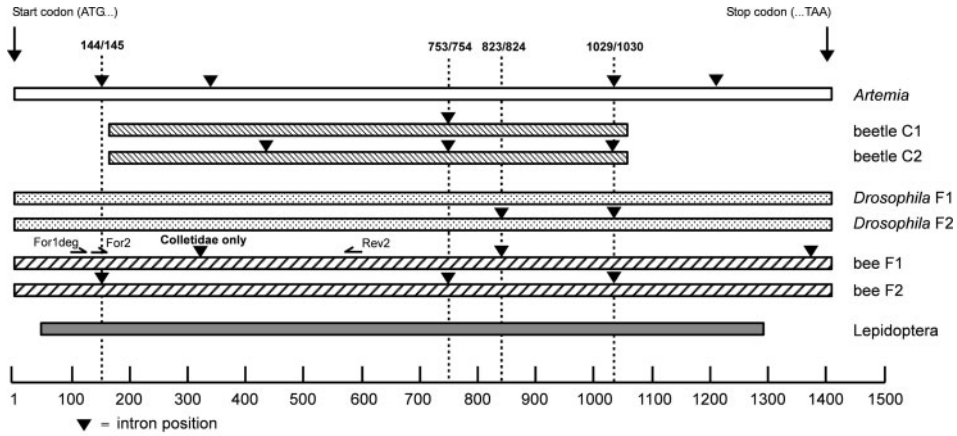

FIG. 1.—Intron positions in EF-1α from well-studied arthropod taxa, modified from Danforth and Ji (1998) and Jordal (2002). Bees, beetles, and *Drosophila* possess two paralogous copies of the gene. The map shows only coding regions and includes the maximum length sequenced from each taxon. Known intron locations are marked with triangles. Introns corresponding exactly in position are indicated by vertical dashed lines. The positions of the colletid intron and the second intron in *Artemia* differ by 11 bp. The horizontal arrows above the bee F1 copy mark the annealing positions of PCR primers used in this study.

Our sampling included all seven currently recognized families of bees (all supraspecific bee taxonomy in this paper follows Michener (2000), and each family contained nearly all constituent subfamilies (18 of 21 subfamilies [table S1 in Supplementary Material online lists all species sampled]). This coverage included bees from virtually all continents, including Africa, Australia, Eurasia, North America, and South America. Additionally, within each individual subfamily, our sampling often was quite dense at lower taxonomic levels. We also included members from the close outgroups to bees, the wasp families Crabronidae and Sphecidae (Alexander and Michener 1995), as well as the more distantly related Formicidae (ants).

All sequence and phylogenetic analyses were performed using PAUP* version 4.0b10 (Swofford 2002). To verify that all sequences obtained in this study belong to the F1 homolog of EF-1α, we constructed distance and parsimony trees of our sequences (exons only) combined with the known F1 copy in *Apis mellifera* (GenBank accession number X52884) and F2 copies of this gene from a representative of each bee family (GenBank accession numbers AF015267, AF435383, AY230129, and AY230131 and unpublished data). The distance tree was calculated using Kimura two-parameter distances (Kimura 1980) analyzed under the neighbor-joining clustering algorithm (Saitou and Nei 1987). The parsimony tree was inferred using a heuristic search strategy of 100 random addition replicates of TBR branch swapping. We ran an additional parsimony analysis using only bee F1 sequences. In all cases, we assessed robustness of clades using the nonparametric bootstrap (Felsenstein 1985).

Results

Initial gel electrophoresis results obtained from PCR amplification indicated length variation in some taxa, suggesting the presence of an intron in this region. To verify that these results were not caused by amplification of the paralogous F2 copy (or other loci), we used PCR amplification to directly sequence 56 taxa representative of the diversity of bees and their immediate outgroups (see table S1 in Supplementary Material online). Phylogenetic analyses of the exon sequences (427 bp) obtained from all taxa indicate that all nominal F1 sequences form a group to the exclusion of any included F2 copy with 100% bootstrap support in both parsimony and distance analyses (see figure S1 in Supplementary Material online), implying orthology of all F1 sequences gathered in this study.

Alignment of these sequences and comparison with *Apis mellifera* indicate the presence of an intron in all 12 species of Colletidae surveyed (fig. 2). This intron is absent from all other species of bees (37 spp.), wasps (four spp.), and ants (three spp.) sampled. The colletid intron occurs at position 313/314 relative to the *Apis mellifera* coding sequence (fig. 1). The alignment reveals strict conservation of the intron splice site (fig. 2); we find no evidence of intron sliding. The initial and final 2 bp of all intron sequences match the canonical GT/AG template for spliceosomal introns (Lewin 2000). The intron is inserted in symmetrical phase 1 (i.e., between codon positions 1 and 2). The AT content is much higher in intron sequences (64%) compared with the surrounding exon sequences (44%), a condition commonly reported (Csank, Taylor, and Martindale 1990; Kwiatowski, Skarecky, and Ayala 1992). However, there is no substantial difference in exon AT content between taxa with introns (43%) compared with those without introns (44%).

Sequence length of the intron varies widely among colletid taxa, ranging from 101 to 1,044 bp (fig. 2). The majority of intron sequences, however, fall in the range of 200 to 400 bp. Considerable variation in intron length exists even within the single genus *Colletes*, which contains species with the longest intron sampled (1,044 bp), the shortest (101 bp), as well as one of intermediate length (363 bp). In an effort to examine phylogenetic patterns of intron length evolution, we conducted parsimony analysis of all bee F1 EF-1α exon sequences gathered in this study. Analysis of this small data set resulted in 96 most-parsimonious trees, the strict consensus of which is shown in figure S2 of Supplementary Material

online. Although the consensus tree recovers colletid monophyly and suggests that Stenotritidae is the sister group to the Colletidae, neither result receives strong bootstrap support. Furthermore, relationships within Colletidae remain largely unresolved, preventing analysis of trends in intron length evolution with the current data.

Discussion

We discovered the presence of an intron in EF-1α (F1 copy) unique to the bee family Colletidae. This intron is present in all 12 colletid species sampled in this study. This sampling included four (Colletinae, Euryglossinae, Hylaeinae, and Xeromelissinae) of the five subfamilies of Colletidae. We were unable to amplify this gene from specimens in the fifth subfamily Diphaglossinae, either because of primer mismatch or because of a long intron. These attempts included the design of alternative primers and cloning of faint amplification products, which turned out to be clearly nonhomologous sequences (results not shown). Because we never witnessed any amplification band consistent in length with an intronless copy, we consider it likely that the intron is also present in this subfamily, but we cannot verify this at the present time.

We found that this intron is absent from all other bees in our study. Our sampling broadly covered the taxonomic diversity of bees, suggesting the complete absence of this intron from all noncolletid bees. This conclusion is consistent with two recent phylogenetic studies (Leys, Cooper, and Schwarz 2002; Schwarz, Bull, and Cooper 2003) that report sequences from 59 species in 12 genera from the bee subfamily Xylocopinae (see table S2 in Supplementary Material online), all of which lack this intron. This intron also is absent from all wasp and ants sampled in the current study, and does not occur in *Drosophila* (Hovemann et al. 1988) or beetles (Jordal 2002). Furthermore, an intron is absent from the equivalent position in species from other arthropod orders with only a single reported EF-1α copy, including Collembola (Carapelli et al. 2000), Diplura (Carapelli et al. 2000), Hemiptera (aphids [Normark 1999; von Dohlen, Kurosu, and Aoki 2002]), Lepidoptera (Cho et al. 1995; Mitchell et al. 1997), and Odonata (damselflies [Jordan, Simon, and Polhemus 2003]). The fact that this intron is known to be absent from so many arthropod lineages provides strong evidence that its presence only in colletid bees is the result of a comparatively recent insertion event.

Another intron has been documented in a position 11 bp downstream from that in Colletidae (based on sequence alignment [results not shown]) in the following taxa: *Artemia* (brine shrimp) (Lenstra et al. 1986), *Scutigera* (myriapod), *Acerentomon* (proturan), and several genera of Collembola (final three groups from Carapelli et al. [2000]). Although intron sliding has been invoked by some to explain slightly discordant intron positions between taxa, this phenomenon has been very difficult to demonstrate for putative sliding events greater than 1 bp to several bp (Stoltzfus et al. 1997; Sato et al. 1999; Rogozin, Lyons-Weiler, and Koonin 2000). Because of the larger positional discrepancy of the apterygotan intron and its complete absence from all pterygote insects sampled, we interpret it as a nonhomologous intron whose position is coincidentally close to the intron found in Colletidae.

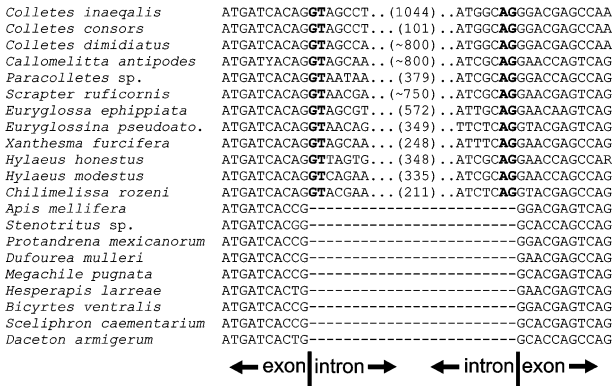

FIG. 2.—Nucleotide sequence alignment of PCR amplified sections of EF-1α (F1 copy) from 12 species from the bee family Colletidae and representatives from other bee, wasp, and ant families. Presence of the intron is restricted to members of Colletidae. Only regions immediately surrounding the exon/intron boundaries are shown, with the initial GT and final AG of each intron indicated by boldface. Dots indicate intervening intron sequences (of varying lengths) between the splicing sites. The total length of each intron is recorded in parentheses. Dashes indicate alignment gaps.

Colletidae is a diverse family containing over 2,000 species that, along with the family Stenotritidae (21 species endemic to Australia), traditionally have been considered the most "primitive" bees (Michener 1944, 1979, 2000). In the most recent morphological analysis of bee family relationships (Alexander and Michener 1995), their phylogenetic affinities were not well established. The comparative distribution of the intron reported in this study provides a new character supporting monophyly of the colletid bees. Furthermore, some authors argue that stenotritids are derived from within the Colletidae (McGinley 1981; Alexander and Michener 1995; Engel 2001), whereas others contend that Stenotritidae are a distinct group based on glossal morphology (McGinley 1980), nesting biology (Houston 1975), and embryology (Torchio 1984). Our results support the latter hypothesis because stenotritids lack this unique intron (fig. 3).

Several previous studies also have demonstrated the utility of recent intron insertions in characterizing monophyletic groups. Insertion of a novel intron in the triosephosphate isomerase (*Tpi*) gene is shared by the closely related mosquito genera *Culex* and *Aedes*, to the exclusion of *Anopheles* mosquitoes and other organisms surveyed (Kwiatowski et al. 1995). Two separate intron gains within the xanthine dehydrogenase (*Xdh*) gene support specific clades within *Drosophila* (Tarrío, Rodríguez-Trelles, and Ayala 1998). Dasyurid marsupials share a novel intron in the mammalian sex-determining gene (*SRK*), which is absent from other mammals (O'Neill et al. 1998). In a survey of intron distributions within six genes, Venkatesh, Ning, and Brenner (1999) discovered that in several cases, higher-level fish taxa are characterized by the unique presence of introns.

The possibility of parallel insertions or multiple losses warrants caution when using comparative intron distributions either to argue for recent, unique insertion events or

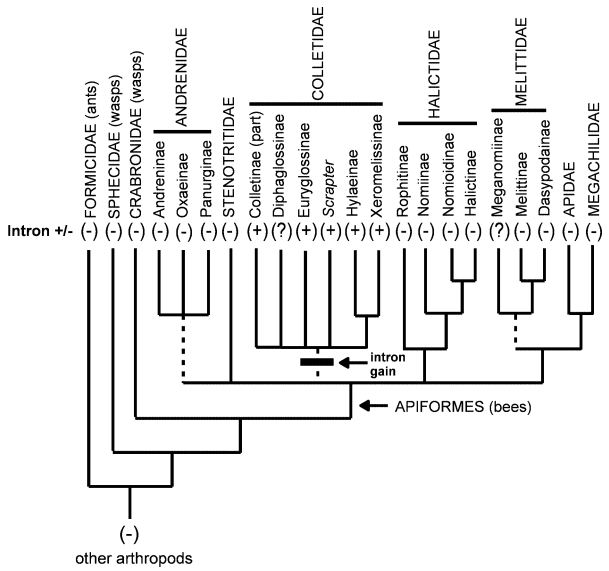

FIG. 3.—Summary of phylogenetic results for bee families and subfamilies based on adult morphology (Alexander and Michener 1995), with sphecid outgroup relationships based on Melo (1999). Dashed branches indicate clades receiving poor or ambiguous support. Symbols in parentheses denote presence (+), absence (−), or unknown status (?) of the F1 EF-1α intron documented in this study. The solid bar marks the inferred point of origin for this intron.

to use those distributions for phylogenetic information. Parallel insertions evidently are rare, with the first unambiguous case recently being uncovered between *Drosophila* and plants (Tarrío, Rodríguez-Trelles, and Ayala 2003). Intron deletion can threaten the utility of using intron insertions for phylogenetic information in cases where such deletion is not recognized as a secondary event (e.g., Krzywinski and Besansky 2002; Wada et al. 2002). Some proponents of the introns-early hypothesis maintain that intron loss in general may be more common than intron gain. For example, genomic comparisons among human, mouse, and rat intron distributions show six clear-cut cases of intron loss but none of intron gain (Roy, Fedorov, and Gilbert 2003). Asymmetrical intron positions in unicellular organisms have also been interpreted as indicative of predominant intron loss (Mourier and Jeffares 2003). On the other hand, phylogenetic analyses of 205 intron positions in a variety of organisms reveal that 77% show a pattern consistent with a single intron gain with no subsequent loss (Stoltzfus et al. 1997). On a more detailed level, comparisons of three genes involved in recombination in plants, animals, and fungi suggest that insertions occur three times more often than deletions (Hartung, Blattner, and Puchta 2002). The present uncertainty over the relative rates of intron gain versus loss mandates thorough taxonomic coverage of an intron distribution before it is used as evidence of a unique insertion event, a requirement we believe we have fulfilled in this case.

We cannot as of yet precisely determine the origination date of this intron insertion. The fossil representation of Colletidae is limited to two species from early Miocene Dominican amber, which may be relatively derived taxa (Michener and Poinar 1996; Engel 1999). Date estimates for the origin of bees place an upper bound at approximately 140 to 120 MYA (Grimaldi 1999; Engel 2001). Given this poor fossil representation and the currently poor phylogenetic resolution of bee families and subfamilies, a more precise age estimate for the origin of this intron will have to await future application of fossil-calibrated molecular dating techniques (e.g., Sanderson 2002; Thorne and Kishino 2002) on a comprehensive, robust bee phylogeny.

Supplementary Material

All bee and wasp voucher specimens are deposited in the Cornell University Insect Collection. All new nucleotide sequences reported in this article have been deposited in GenBank under accession numbers AY362984 to AY363042. These accession numbers are also listed for each species in table S1. Tables S1 and S2 and figures S1 and S2 are available as Supplementary Material online.

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Literature Cited

Alexander, B. A., and C. D. Michener. 1995. Phylogenetic studies of the families of short-tongued bees (Hymenoptera: Apoidae). Univ. Kans. Sci. Bull. **55**:377–424.

Carapelli, A., F. Frati, F. Nardi, R. Dallai, and C. Simon. 2000. Molecular phylogeny of the apterygotan insects based on nuclear and mitochondrial genes. Pedobiology **44**:361–373.

Cavalier-Smith, T. 1991. Intron phylogeny: a new hypothesis. Trends Genet. **7**:145–148.

Cho, S., A. Mitchell, J. C. Regier, C. Mitter, R. W. Poole, T. P. Friedlander, and S. Zhao. 1995. A highly conserved nuclear gene for low-level phylogenetics: elongation factor 1-alpha recovers morphology-based tree for heliothine moths. Mol. Biol. Evol. **12**:650–656.

Csank, C., F. M. Taylor, and D. W. Martindale. 1990. Nuclear pre-mRNA introns: analysis and comparison of intron sequences from *Tetrahymena thermophila* and other eukaryotes. Nucleic Acids Res. **18**:5133–5141.

Danforth, B. N. 1999. Phylogeny of the bee genus *Lasioglossum* (Hymenoptera: Halictidae) based on mitochondrial COI sequence data. Syst. Entomol. **24**:377–393.

Danforth, B. N., L. Conway, and S. Ji. 2003. Phylogeny of eusocial *Lasioglossum* reveals multiple losses of eusociality within a primitively eusocial clade of bees (Hymenoptera: Halictidae). Syst. Biol. **52**:23–36.

Danforth, B. N., and S. Ji. 1998. Elongation factor-1α occurs as two copies in bees: implications for phylogenetic analysis of EF-1α sequences in insects. Mol. Biol. Evol. **15**:225–235.

de Souza, S. J., M. Long, R. J. Klein, S. Roy, S. Lin, and W. Gilbert. 1998. Toward a resolution of the introns early/late debate: Only phase zero introns are correlated with the

structure of ancient proteins. Proc. Natl. Acad. Sci. USA **95**:5094–5099.
Doolittle, W. F. 1978. Genes in pieces: were they ever together? Nature **272**:581–582.
Engel, M. S. 1999. A new xeromelissine bee in Tertiary amber of the Dominican Republic (Hymenoptera: Colletidae). Entomol. Scand. **30**:453–458.
———. 2001. A monograph of the Baltic amber bees and the evolution of the Apoidea (Hymenoptera). Bull. Am. Mus. Nat. Hist. **259**:1–192.
Fedorov, A., A. F. Merican, and W. Gilbert. 2002. Large-scale comparison of intron positions among animal, plant, and fungal genes. Proc. Natl. Acad. Sci. USA **99**:16128–16133.
Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution **39**:783–791.
Gilbert, W. 1979. Introns and exons: Playgrounds of evolution. Academic Press, New York.
Grimaldi, D. A. 1999. The co-radiations of insects and angiosperms in the Cretaceous. Ann. Missouri Bot. Gard. **86**:373–406.
Hankeln, T., H. Friedl, I. Ebersberger, J. Martin, and E. R. Schmidt. 1997. A variable intron distribution in globin genes of *Chironomus*: evidence for recent intron gain. Gene **205**:151–160.
Hartung, F., F. R. Blattner, and H. Puchta. 2002. Intron gain and loss in the evolution of the conserved eukaryotic recombination machinery. Nucleic Acids Res. **30**:5175–5181.
Houston, T. F. 1975. Nests, behavior and larvae of the bee *Stenotritus pubescens* (Smith) and behavior of some related species (Hymenoptera: Apoidea: Stentotrinae). J. Aust. Entomol. Soc. **14**:145–154.
Hovemann, B., S. Richter, U. Walldorf, and C. Cziepluch. 1988. Two genes encode related cytoplasmic elongation factors 1α (EF-1α) in *Drosophila melanogaster* with continuous and stage specific expression. Nucleic Acids Res. **16**:3175–3194.
Jordal, B. H. 2002. Elongation Factor 1α resolves the monophyly of the haplodiploid ambrosia beetles Xyleborini (Coleoptera: Curculionidae). Insect Mol. Biol. **11**:453–465.
Jordan, S., C. Simon, and D. Polhemus. 2003. Molecular systematics and adaptive radiation of Hawaii's endemic damselfly genus *Megalagrion* (Odonata: Coenagrionidae). Syst. Biol. **52**:89–109.
Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. **16**:111–120.
Krzywinski, J., and N. J. Besansky. 2002. Frequent intron loss in the *white* gene: a cautionary tale for phylogeneticists. Mol. Biol. Evol. **19**:362–366.
Kwiatowski, J., M. Krawczyk, M. Kornacki, K. Bailey, and F. J. Ayala. 1995. Evidence against the exon theory of genes derived from the triose-phosphate isomerase gene. Proc. Natl. Acad. Sci. USA **92**:8503–8506.
Kwiatowski, J., D. Skarecky, and F. J. Ayala. 1992. Structure and sequence of the Cu,Zn Sod gene in the Mediterranean fruit fly, *Ceratitis capitata*: Intron insertion/deletion and evolution of the gene. Mol. Phylogenet. Evol. **1**:72–82.
Lenstra, J. A., A. Van Vliet, A. C. Arnberg, F. J. Van Hemert, and W. Möller. 1986. Genes coding for the elongation factor EF-1α in *Artemia*. Eur. J. Biochem. **155**:475–483.
Lewin, B. 2000. Genes VII. Oxford University Press, Oxford, UK.
Leys, R., S. J. B. Cooper, and M. P. Schwarz. 2002. Molecular phylogeny and historical biogeography of the large carpenter bees, genus *Xylocopa* (Hymenoptera: Apidae). Biol. J. Linn. Soc. **77**:249–266.
Logsdon, J. M., Jr. 1998. The recent origins of spliceosomal introns revisited. Curr. Opin. Genet. Dev. **8**:637–648.
Lynch, M., and A. O. Richardson. 2002. The evolution of spliceosomal introns. Curr. Opin. Genet. Dev. **12**:701–710.
Maroni, G. 1993. An atlas of *Drosophila* genes. Oxford University Press, Oxford, UK.
McGinley, R. J. 1980. Glossal morphology of the Colletidae and recognition of the Stenotritidae at the family level. J. Kans. Entomol. Soc. **53**:539–552.
———. 1981. Systematics of the Colletidae based on mature larvae with phenetic analysis of apoid larvae, Hymenoptera: Apoidea. Univ. Calif. Publ. Entomol. **91**:i–xvi, 1–307.
Melo, G. A. R. 1999. Phylogenetic relationships and classification of the major lineages of Apoidea (Hymenoptera), with emphasis on the crabronid wasps. Sci. Pap. Nat. Hist. Mus. Univ. Kans. **14**:1–55.
Michener, C. D. 1944. Comparative external morphology, phylogeny, and a classification of the bees. Bull. Am. Mus. Nat. Hist. **82**:151–326.
———. 1979. Biogeography of the bees. Ann. Missouri Bot. Gard. **66**:277–347.
———. 2000. The bees of the world. Johns Hopkins University Press, Baltimore, Md.
Michener, C. D., and G. Poinar, Jr. 1996. The known bee fauna of the Dominican amber. J. Kans. Entomol. Soc. **69**:353–361.
Mitchell, A., S. Cho, J. C. Regier, C. Mitter, R. W. Poole, and M. Mathews. 1997. Phylogenetic utility of elongation factor-1α in Noctuoidae (Insecta: Lepidoptera): the limits of synonymous substitution. Mol. Biol. Evol. **14**:381–390.
Mourier, T., and D. C. Jeffares. 2003. Eukaryotic intron loss. Science **300**:1393.
Normark, B. B. 1999. Evolution in a putatively ancient asexual aphid lineage: recombination and rapid karyotype change. Evolution **53**:1458–1469.
O'Neill, R. J. W., F. E. Brennan, M. L. Delbridge, R. H. Crozier, and J. A. M. Graves. 1998. De novo insertion of an intron into the mammalian sex determining gene, *SRY*. Proc. Natl. Acad. Sci. USA **95**:1653–1657.
Palmer, J. D., and J. M. Logsdon, Jr. 1991. The recent origin of introns. Curr. Opin. Genet. Dev. **1**:470–477.
Rogers, J. H. 1990. The role of introns in evolution. FEBS Lett. **268**:339–343.
Rogozin, I. B., J. Lyons-Weiler, and E. V. Koonin. 2000. Intron sliding in conserved gene families. Trends Genet. **16**:430–432.
Roy, S. W., A. Fedorov, and W. Gilbert. 2003. Large-scale comparison of intron positions in mammalian genes shows intron loss but no gain. Proc. Natl. Acad. Sci. USA **100**:7158–7162.
Saitou, N., and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4**:406–425.
Sanderson, M. J. 2002. Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. Mol. Biol. Evol. **19**:101–109.
Sato, Y., T. Niimura, K. Yura, and M. Go. 1999. Module-intron correlation and intron sliding in family F/10 xylanase genes. Gene **238**:93–101.
Schwarz, M. P., N. J. Bull, and S. J. B. Cooper. 2003. Molecular phylogenetics of allodapine bees, with implications for the evolution of sociality. Syst. Biol. **52**:1–14.
Stoltzfus, A., J. M. Lodsdon, Jr., J. D. Palmer, and W. F. Doolittle. 1997. Intron "sliding" and the diversity of intron positions. Proc. Natl. Acad. Sci. USA **94**:10739–10744.
Swofford, D. L. 2002. PAUP*: phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer Associates, Sunderland, Mass.
Tarrío, R., F. Rodríguez-Trelles, and F. J. Ayala. 1998. New *Drosophila* introns originate by duplication. Proc. Natl. Acad. Sci. USA **95**:1658–1662.

———. 2003. A new *Drosophila* spliceosomal intron position is common in plants. Proc. Natl. Acad. Sci. USA **100**:6580–6583.

Thorne, J. L., and H. Kishino. 2002. Divergence time and evolutionary rate estimation with multilocus data. Syst. Biol. **51**:689–702.

Torchio, P. F. 1984. The nesting biology of *Hylaeus bisinuatus* Forster and development of its immature forms (Hymenoptera: Colletidae). J. Kans. Entomol. Soc. **57**:276–297.

Venkatesh, B., Y. Ning, and S. Brenner. 1999. Late changes in spliceosomal introns define clades in vertebrate evolution. Proc. Natl. Acad. Sci. USA **96**:10267–10271.

von Dohlen, C. D., U. Kurosu, and S. Aoki. 2002. Phylogenetics and evolution of the eastern Asian-eastern North American disjunct aphid tribe, Hormaphidini (Hemiptera: Aphididae). Mol. Phylogenet. Evol. **23**:257–267.

Wada, H., M. Kobayashi, R. Sato, N. Satoh, H. Miyasaka, and Y. Shirayama. 2002. Dynamic insertion-deletion of introns in deuterostome EF-1α genes. J. Mol. Evol. **54**:118–128.

Walldorf, U., and B. T. Hovemann. 1990. *Apis mellifera* cytoplasmic elongation factor 1-alpha (EF-1alpha) is closely related to *Drosophila melanogaster* EF-1alpha. FEBS Lett. **267**:245–249.

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