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ORIGINS, EVOLUTION, AND DIVERSIFICATION OF CLEPTOPARASITIC LINEAGES IN LONG-TONGUED BEES

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The evolution of parasitic behavior may catalyze the exploitation of new ecological niches yet also binds the fate of a parasite to that of its host. It is thus not clear whether evolutionary transitions from free-living organism to parasite lead to increased or decreased rates of diversification. We explore the evolution of brood parasitism in long-tongued bees and find decreased rates of diversification in eight of 10 brood parasitic clades. We propose a pathway for the evolution of brood parasitic strategy and find that a strategy in which a closed host nest cell is parasitized and the host offspring is killed by the adult parasite represents an obligate first step in the appearance of a brood parasitic lineage; this ultimately evolves into a strategy in which an open host cell is parasitized and the host offspring is killed by a specialized larval instar. The transition to parasitizing open nest cells expanded the range of potential hosts for brood parasitic bees and played a fundamental role in the patterns of diversification seen in brood parasitic clades. We address the prevalence of brood parasitic lineages in certain families of bees and examine the evolution of brood parasitism in other groups of organisms.

KEY WORDS: **Behavioral innovation, convergent evolution, cuckoo bees, Emery's Rule, modes of cleptoparasitism.**

Parasitism may be defined as a life-history strategy in which "an organism lives in or on another living organism, obtains from it part or all of its organic nutriment, commonly exhibits some degree of adaptive structural modification, and causes some degree of real damage to its host" (Price 1980). Parasitism may take many forms, including ectoparasitism and endoparasitism, parasitoidism, and brood parasitism, and has evolved numerous times independently throughout the tree of life. The evolution of a parasitic lineage from nonparasitic ancestors implies dramatic changes in physiology, phenology, behavior, and larval diet. Although such changes may facilitate the exploitation of new ecological niches, the evolution of a parasitic life-history strategy also intimately binds the fate of a parasite to that of its host (Price 1980; Krüger et al. 2009). Thus, the macroevolutionary consequences of a parasitic lifestyle on diversification may in fact be twofold. Some groups, such as the megadiverse parasitoid Hymenoptera and the parasitic Platyhelminthes, appear to have undergone remarkable radiations (Littlewood et al. 1999; Whitfield 2003, and references therein), possibly as a result of diversification into "adaptive zones" or diversifying selection following

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extreme ecological specialization (Wiegmann et al. 1993). Other groups, however, show the contrary: a comparison of diversification rates among carnivorous parasites suggests that a parasitic life-history strategy may in fact be an evolutionary "dead end" accompanied by an increased risk of extinction (Wiegmann et al. 1993).

Brood parasitism is a unique form of parasitism in which the parasite usurps the nest of its host and often kills its offspring; this behavior has been reported in diverse organisms, including solitary wasps (Evans 1953; O'Neill 2001), dung beetles (Trumbo 1994), fish (Sato 1986), and birds (Sorenson and Payne 2002). Multiple lineages of bees are obligate brood parasites; although some of these lineages appear to be ancient and highly species-rich, hinting at possible radiations, others are relatively species-poor (Rozen 2003; Michener 2007). Bees are thus an ideal group for exploring questions related to the effects of parasitism on macroevolutionary processes. Like cuckoo birds, brood parasitic bees (also referred to as "cleptoparasites," a term that will be used hereafter) neither build nor provision their own nests but instead deposit their eggs in nests that are built and provisioned by a host belonging to a different species. Although the offspring of avian brood parasites are progressively fed by a host, cleptoparasitic bee larvae develop on the pollen mass provisioned by the host bee for her own offspring and thus never come into direct contact with the adult host.

Nest-building solitary bees provision their nest cells with just enough pollen to sustain the growth of a single larva (Müller et al. 2006). The provisions in one brood cell, therefore, are insufficient to nourish both the host larva and any cleptoparasitic larvae that may be present in the same nest cell (Wcislo 1987). To eliminate the competition for resources presented by the host's offspring, cleptoparasitic bees have evolved several strategies, which we broadly divide into three main categories.

In the first, the adult female cleptoparasite enters a closed nest cell (i.e., a nest cell that has already been provisioned and sealed shut) and removes or kills the host eggs and larvae (herein referred to as "AC strategy," short for "adult-closed"). This strategy is exhibited by the megachilid genus *Euaspis* and the apid genus *Exaerete* (Bennett 1972; Iwata 1976; see Table 1 for other examples). In the second strategy, the adult female cleptoparasite opens the closed nest cell of a host bee, deposits her own egg in the cell, and then recloses the cell. In this case, however, it is not the adult female but rather her larva that kills the host's offspring (herein referred to as "LC strategy," short for "larva-closed"). Such larvae are referred to as "hospicidal" (Rozen 1989): one or more instars are highly aggressive and armed with modified, sharp mandibles which they use to kill the eggs or larvae of their host, as well as those of any other cleptoparasites also present in the cell. The LC strategy is observed, for example, in some members of the megachilid tribe Dioxyini and in the apid genus *Melecta* (Rozen and Favreau 1967; Rozen 1991; Rozen 2003; Table 1). The third mode of cleptoparasitism is similar to the second except that the adult female deposits her egg in a nest cell that is still in the process of being provisioned by the host (i.e., an open nest cell); as in the second mode, a hospicidal larval instar kills all other eggs and larvae present in the cell (herein referred to as "LO strategy," short for "larva-open"). This strategy is observed in multiple lineages, including the megachilid genus *Coelioxys* and the apid genus *Nomada* (Rozen 1991; Rozen and Kamel 2009; Rozen et al. 2010, and all references therein; Table 1).

Although cleptoparasitic strategy is usually highly conserved within genera and tribes, several clades within the families Apidae and Megachilidae exhibit more than one mode of cleptoparasitism. These two families together comprise the long-tongued (L-T) bees, a group that includes some 10,000 species (one half of all bee species), approximately 20% of which are cleptoparasites (Michener 2007). A recent phylogenetic analysis demonstrated four independent origins of cleptoparasitism in Apidae and found that 99% of apid cleptoparasites belong to a single clade (Cardinal et al. 2010). Although 10 independent origins of cleptoparasitism are currently postulated in the family Megachilidae (Michener 2007), this number has never been verified using phylogenetic inference.

In this study, we use maximum likelihood- and Bayesian-based methods and a large, multilocus data set to reconstruct the evolutionary origins of cleptoparasitism in the bee family Megachilidae. Using the L-T bees as a model group, we trace the evolution of cleptoparasitic strategy, explore its impact on species diversification in cleptoparasitic clades, and discuss the forces driving transitions in cleptoparasitic strategy. We use our results to propose a hypothesis for the evolution of cleptoparasitic strategy and to examine the evolution of cleptoparasitic strategy in other groups of aculeate hymenoptera.

Materials and Methods

TAXON SAMPLING

For molecular phylogenetic analysis, we chose 190 ingroup taxa representing a broad taxonomic sample of L-T bees and eight outgroup taxa representing the remaining families of bees (excluding Stenotritidae). We included 118 species representing all seven tribes from the family Megachilidae and 72 species representing 32 of the 33 tribes from the family Apidae (the tribe Caenoprosopidini was not included in our analysis); we sampled densely within cleptoparasitic lineages for both families. One outgroup species was taken from each of the families Colletidae, Andrenidae, Halictidae, and from each of the principle lineages of the purportedly paraphyletic Melittidae (one outgroup species each from Meganomiinae and Dasypodainae and three species

Table 1. Ten independent origins of cleptoparasitism in long-tongued bees. For each group, we list the crown age (where possible), stem age, stem group diversification rate ($e = 0.9$), clade size, strategy, character coding for Bayes Traits analysis, known hosts and corresponding references. Value in parentheses after stem group diversification rate for each origin represents the stem group diversification rate for the group in which each origin is nested. We use Megachilinae sensu Gonzalez et al. 2012; the "large apid cleptoparasitic clade" = Nomadinae + the ericrocidine line + Melectini. Crown and stem ages taken from BEAST analyses.

Ten origins of cleptoparasitism	Major clade	Genus/tribe	Crown age (million years; (95% CI)	Stem age (million years; 95% CI)	Stem group diversification rate ($e = 0.9$)	Clade size	Strategy	Coding Bayes Traits	Host/s	Reference
Megachilidae										
1		Dioxyini	31.1 (18.9–46.7)	78 (57.5–97.6)	0.019 (Megachilinae = 0.0602)	35	LC, LO	1 (*Dioxys pomonae*), 12 (the rest)	Osmiini, Anthidiini, Megachilini (Megachilidae)	Rozen and Favreau 1967; Westrich 1989; Rozen and Özbek 2005
2		*Coelioxys*	16.8 (12.1–22.2)	21.5 (16.7–26.6)	0.183 (*Megachile* = 0.11)	311	LO	2	Mostly *Megachile* (Megachilidae); also *Centris*, *Anthophora* and *Euglossa* (Apidae)	Gonzalez et al. 2007; Rozen et al. 2010, and references therein
3		*Radoszkowskiana*	–	30.2 (24.4–36.4)	0.00869 (*Megachile* = 0.11)	4	Likely LO	12	*Megachile* (Megachilidae)	Rozen et al. 2010, and references therein
4	*Stelis* clade		31.2 (25.1–38.2)	38.6 (31.3–46.5)	0.0551 (Anthidiini = 0.0514)					
		Afrostelis	–	25.4 (18.3–33.0)		5	Unknown	12	*Heriades* (Megachilidae)	Taylor 1962
		Euaspis	10.2 (5.5–15.5)	25.4 (18.3–33.0)		12	AC	1	*Megachile* (*Callomegachile*), *Megachile* (*Gronoceras*) (Megachilidae)	Iwata 1976
		Stelis (*Stelidomorpha*)	–	22.6 (15.0–30.3)		3	–[1]	1	*Megachile* (*Chalicodoma*) (Megachilidae)	Fabre 1914
		Stelis rozeni	–	22.6 (15.0–30.3)		–	Unknown	12	unknown	-
		Stelis (*Protostelis*)	–	19.1 (15.1–23.9)		1	Unknown	12	*Anthidiellum strigatum* (Megachilidae)	Westrich 1989
		Stelis (*Dolichostelis*)	9.1 (4.7–14.1)	19 (13.1–24.7)		6	AC	1	*Megachile* (*Chelostomoides*) (Megachilidae)	Parker et al. 1987
		Stelis (*Malanthidium*)	10.5 (5.7–15.8)	22.1 (17.6–27.2)		2	Unknown	12	unknown	-
		Stelis (*Stelis*)	15.2 (12.0–18.9)	19.1 (15.1–23.9)		75	LO	2	Members of Osmiini, Anthidiini, Megachilini, Lithurgini (Megachilidae)	Rust and Thorp 1973; Rozen 1987; Westrich 1989; Rozen and Kamel 2009
5		*Austrostelis* +*Hoplostelis*	13.3 (7.1–20.6)	25.4 (18.4–34.3)	0.0292 (Anthidiini = 0.0514)					
		Austrostelis		13.3 (7.1–20.6)		8	Unknown	12	*Epanthidium* (Megachilidae)	Zanella and Ferreira 2005
		Hoplostelis		13.3 (7.1–20.6)		4	AC	1	*Euglossa* (Apidae)	Bennett 1966
6		*erythrogastra* group of *Hoplitis* (*Hoplitis*)	1.9 (0.8–3.7)	11.9 (7.6–16.5)	0.022 (*Hoplitis* = 0.0984)	4	Unknown	12	members of *Hoplitis* (*Hoplitis*) (Megachilidae)	Mavromoustakis 1954; Warncke 1991; Sedivy et al. 2013a,b

(Continued)

Table 1. Continued.

Ten origins of cleptoparasitism	Major clade	Genus/tribe	Crown age (million years; (95% CI)	Stem age (million years; 95% CI)	Stem group diversification rate ($e = 0.9$)	Clade size	Strategy	Coding Bayes Traits	Host/s	Reference
Apidae										
7		*Aglae*	–	30.9 (21.6 –42.0)	0 (Euglossini = 0.0327)	1	Unknown	12	*Eulaema* (Apidae)	Michener 2007
8		*Exaerete*	7.9 (3.6–13.2)	27.1 (18.3–38.5)	0.015 (Euglossini = 0.0327)	6	AC, possibly LC	1	*Eulaema, Eufriesea* (Apidae)	Bennett 1972; Garofalo and Rozen 2001
9		*Ctenoplectrina*	–	29 (13.8–49.8)	0.00329 (Ctenoplectrini = 0.0119)	2	unknown	12	likely *Ctenoplectra* (Apidae)	Schaefer and Renner 2008
10	Large apid cleptoparasitic clade		111.9 (95.6–129.5)	128.8 (112.5–146.6)	0.0396 (Apidae = 0.0431)					
	Melectine clade									
		Melectini	53.2 (35.8–73.2)	111.9 (95.6–129.5)		211	LC	1	Anthophorini, Eucerini (Apidae)	Rozen 1969a,1969b; Wafa and Mohamed 1970; Michener 2007 and references therein
	Ericrocidine clade		95.3 (79.4–113.5)	107.1 (91.2–124.5)						
		Rhathymini	19.6 (9.3–33.3)	75.7 (59.4–93.6)		10	LC	1	*Epicharis* (Apidae)	Rozen 1969b; Michener 2007 and references therein
		Ericrocidini	39.8 (27.6–53.0)	75.7 (59.4–93.6)		53	LC	1	*Centris, Epicharis* (Apidae)	Rozen 1969b; Michener 2007 and references therein
		Osirini (paraphyletic)	n/a	n/a		39	LC[2]	Osiris, Parepeolus: 12 Epeoloides: 2	Tapinotaspidini (Apidae); *Macropis* (Melittidae)	Michener 2007 and references therein
		Coelioxoides	–	79.3 (57.7–99.8)		3	LC	1	*Tetrapedia* (Apidae)	Alves-dos-Santos et al. 2002
		Isepeolini	29 (16.0–44.0)	65.8 (49.6–83.4)		21	likely LO	2	*Colletes,* possibly *Mourecotelles* (Colletidae); *Canephorula* (Apidae)	Michener 1957; Michener 2007 and references therein
		Protepeolini	–	54.1 (37.4–72.0)		5	LO	2	*Diadasia, Melitoma*, and *Ptilothrix* (Apidae)	Rozen, Eickwort, and Eickwort 1978; Roig-Alsina and Rozen 1994

(Continued)

Table 1. Continued.

Ten origins of cleptoparasitism	Major clade	Genus/tribe	Crown age (million years; (95% CI)	Stem age (million years; 95% CI)	Stem group diversification rate ($e = 0.9$)	Clade size	Strategy	Coding Bayes Traits	Host/s	Reference
	Nomadinae		92.9 (77.2–109.7)	107.1 (91.2–124.5)						
		Ammobatini	46.2 (27.5–69.0)	92.9 (77.2–109.7)		95	LO	2	*Ancyla, Tetraloniella, Anthophora* (Apidae); members of Panurginae (Andrenidae); *Nomioides*, some Nomiinae (Halictidae); *Scrapter* (Colletidae)	Rozen and Michener 1968; Michener 2007, and references therein
		Ammobatoidini	54 (36.5–72.2)	69.5 (54.4–85.5)		28	LO	2	*Melitturga, Meliturgula, Calliopsis, Camptopoeum, Pseudopanurgus* and *Protandrena* (Andrenidae)	Rozen 1965; Hurd and Linsley 1972; Michener 2007, and references therein
		Epeolini	49.5 (35.1–63.1)	71 (55.7–86.3)		311	LO	2	various members of Eucerini, Anthophorini, Centridini, Emphorini (Apidae); *Colletes, Ptiloglossa* (Colletidae); *Oxaea, Protoxaea* (Andrenidae); *Dieunomia* (Halictidae)	Michener 2007, and references therein
		Nomadini	–	75.3 (59.2–91.8)		795	LO	2	mostly *Andrena* (Andrenidae); other hosts include *Agapostemon, Halictus, Lasioglossum,* and *Lipotriches* (Halictidae); *Panurgus* (Andrenidae); *Melitta* (Melittidae); *Colletes* (Colletidae); *Exomalopsis* and *Eucera* (Apidae)	Michener 2007, and references therein
		Townsendiellini	–	42.1 (25.6–59.0)		3	LO	2	*Hesperapis* (Melittidae)	Michener 2007
		Biastini	–	42.1 (25.6–59.0)		11	LO	2	*Protodufourea, Systropha, Rophites, and Dufourea* (Halictidae, Rophitinae)	Rozen et al. 2009 and references therein
		Neolarrini	–	50.9 (35.3–67.2)		14	LO	2	*Perdita, Calliopsis* (Andrenidae)	Rozen 1965; Michener 2007
		Hexepeolini	–	59.9 (44.3-75.7)		1	LO	2	*Ancylandrena* (Andrenidae)	Rozen 1994
		Brachynomadini	38 (23.7–55.1)	71 (55.7–86.3)		26	LO	2	*Exomalopsis, Anthophorula* (Apidae); *Liphanthus, Protandrena, Psaenythia* (Andrenidae); *Leioproctus* (Colletidae)	Michener 2007, and references therein
		Caenoprosopidini	n/a	n/a		2	Unknown	n/a	*Arhysosage, Callonychium* (Andrenidae)	Rozen and Roig-Alsina 1991

[1]**The strategy of *Stelis* (*Stelidomorpha*) is unique among bees: the cleptoparasitic larvae consume all of the provisions intended for the host's offspring, effectively starving them to death (Fabre 1923).**

[2]**The osirine species *Protosiris gigas*, absent from our phylogeny, may be both an AC and LC strategist; *P. gigas* is the only member of Osirini whose strategy is known (Rozen et al. 2006).**

from Melittinae). Collection localities and DNA voucher numbers are listed in Table S1. Voucher specimens are deposited in the Cornell University Insect Collection, at the USDA-ARS Bee Biology and Systematics Laboratory at Utah State University in Logan, Utah, the York University Insect Collection, and at the University of Neuchatel Insect Collection.

DATA SET AND ALIGNMENT

Our data set included a total of 5925 base pairs from four nuclear protein-coding genes and one nuclear ribosomal gene: CAD (sequences for most taxa ~960 base pairs, although some sequences up to 1335 base pairs); NAK (1488 base pairs); LW-rhodopsin (678 base pairs); EF1-alpha, F2 copy (1110 base pairs); and 28S (1314 base pairs). Previously published sequences were downloaded from GenBank: megachilid sequences were taken largely from Litman et al. (2011), whereas apid sequences were mostly obtained from Cardinal et al. (2010). New sequence data were generated following the DNA extraction and sequencing protocols described by Danforth et al. (1999). PCR primers and conditions for all sequences new to this study were identical to those listed in Litman et al. (2011). New sequencing was performed at the Cornell University Life Sciences Core Laboratories Center using an Applied BioSystems 3730xl DNA analyzer and at the University of Neuchatel using an Applied Biosystems 3500 DNA analyzer. Sequences were edited using Sequencher version 5.0 sequence analysis software (Gene Codes Corporation 2011). Protein coding genes were aligned using MAFFT (Katoh et al. 2002) and then adjusted by eye in MacClade (Maddison and Maddison 2005). All introns were excluded. The ribosomal gene 28S was aligned by secondary structure following the method described by Kjer (1995) and using the 28S secondary structure model of *Apis mellifera* (Gillespie et al. 2006); unalignable regions were removed. New sequence data are archived in GenBank and all DNA accession numbers are listed in Table S2.

PARTITIONING REGIME AND MODEL TESTING

We employed a partitioning regime in which the first, second, and third codon positions of each protein-coding gene were grouped together and the stem and loop regions of 28S were each placed in a unique partition, resulting in five partitions. We also explored partitioning regimes in which we grouped codon positions according to their overall substitution rates; although these analyses yielded phylogenetic trees highly congruent with those presented here, multiple parameters failed to reach stationarity, indicating overparameterization.

Best-fit models of nucleotide substitution were selected using MrModelTest version 2.3 (Nylander 2008). Independent model tests were run on each of the data partitions described earlier and the Akaike Information Criterion (AIC; Akaike 1974) was used to estimate the best-fit model of nucleotide substitution. Although the best-fit model for each partition was estimated to be GTR + I + Γ, model parameters under this model failed to reach stationarity in some analyses, even after more than 120 million generations. To reduce the number of parameters estimated from the data, the next most likely model for each partition was chosen from the output of MrModelTest version 2.3; in each case, models included either a parameter for invariant sites (I) or a parameter for among site rate variation (Γ) but not both. Using this modeling regime, parameters for all models converged upon stable values in all analyses. The models used for each partition are as follows: (1) first codon positions: GTR + Γ (general time reversible model with a gamma correction for among site rate variation); (2) second codon positions: GTR + I (general time reversible model with an allowance for a proportion of invariant sites); (3) third codon positions: GTR + Γ; (4) 28S loop region: GTR + Γ; and (5) 28S stem region: GTR + I.

PHYLOGENETIC AND DIVERGENCE DATING ANALYSES

A simultaneous Bayesian phylogenetic and divergence dating analysis was performed using BEAST version 1.6.1 (Drummond and Rambaut 2007) on the Cornell University Computational Biology Service Unit cluster. Analyses were run using an uncorrelated lognormal relaxed clock model with a Yule process tree prior. Models of nucleotide substitution described earlier were implemented for each partition and were unlinked across partitions. We ran 10 independent analyses for between 14 and 30 million generations each, resulting in a total of 162 million generations. After eliminating an appropriate burnin from each analysis, we calculated the maximum clade credibility tree by using TreeAnnotator version 1.7.1 (Drummond and Rambaut 2007) to summarize the results from the posterior distribution of trees generated in our BEAST analysis. Node ages were calculated as mean ages, thus the age of each node in the maximum clade credibility tree represents the mean node age across the entire distribution of trees for any given clade.

We used data taken from the fossil record to time calibrate seven internal nodes on our phylogeny (see Figure S1 for fossil placement). Fossil calibrations are identical to those described in Litman et al. (2011), with the exception of *Paleohabropoda oudardi*, an anthophorine fossil from the French Paleocene (Michez et al. 2009). The analysis we present here includes three extant anthophorine taxa: *Anthophora montana*, *Pachymelus peringueyi*, and *Deltoptila aurulentocaudata* (the analysis of Litman et al. (2011) included only *A. montana* and *P. peringueyi*). The phylogenetic position of *P. oudardi* within the tribe Anthophorini is unclear: a parsimony analysis based on 17 morphological characters places it outside the clade containing the other three Anthophorini, whereas an analysis based on wing morphometrics demonstrates a relationship closer to *Pachymelus* and *Deltoptila*

than to *Anthophora* (Michez et al. 2009). We model this uncertainty in phylogenetic position by using *P. oudardi* to calibrate the node uniting all three anthophorine taxa. We assign this calibration point a normal prior distribution with a mean of 60 million years and a standard deviation of 6.0.

The age of the root node of our phylogeny corresponds to the age of the most recent common ancestor of all bees. We calibrated the root node using a normal prior distribution and used a recent estimate for the age of the bees (Litman et al. 2011) to assign a mean value of 145 million years to this distribution. A standard deviation of 14.5 was chosen such that the 95% confidence interval of this distribution spanned the range of reasonable estimates for the age of the bees (Cardinal et al. 2010; Litman et al. 2011).

Maximum likelihood analyses were performed using RAxML version 7.2.8 (sequential version raxmlHPC; Stamatakis et al. 2005; Stamatakis 2006). We used the rapid bootstrapping algorithm to run 1000 bootstrap replicates using the GTRCAT approximation, followed by a search for the best-scoring maximum likelihood tree under a GTR + Γ model. The partitioning regime was the same as that described earlier.

ANCESTRAL STATE RECONSTRUCTIONS

We performed a maximum likelihood-based analysis using the "Multistate" option in BayesTraits (Pagel 1997, 1999) to reconstruct the evolution of cleptoparasitic strategy in L-T bees and to calculate transition rates between strategies. Preliminary analyses with four different character codings (nest-building, AC, LC, and LO strategies), and thus 12 different transition rates, did not yield results that converged upon stable values, implying overparameterization of this model. Terminal taxa were thus coded as nest-building (0), cleptoparasites of closed nest cells (1), or cleptoparasites of open nest cells (2). Character coding was based on information taken from the relevant literature (Table 1). All known members of the subgenus *Stelis (Stelis*) are cleptoparasites of open nest cells. In cases where the behavior of terminal taxa belonging to this subgenus was unknown, species were coded as (2). In some cases, lineages are known cleptoparasites but the details of their cleptoparasitic strategy are unknown. In these cases, terminals were coded as (1, 2). Analyses were performed using 1000 randomly chosen post-burnin trees from our BEAST analysis. We took a $\Delta \ln L$ of at least two units as evidence for a significant difference between independently run analyses (Pagel 1999).

ANALYSES OF DIVERSIFICATION AND CLADE SPECIES RICHNESS

We used MEDUSA (Alfaro et al. 2009), available as part of the Geiger package in R (Harmon et al. 2008; R-Development Core Team 2012), to test the hypothesis that changes in cleptoparasitic strategy are associated with changes in the tempo of species diversification. Two cleptoparasitic clades within the L-T bees include members exhibiting more than one cleptoparasitic strategy: the *Stelis* clade (Megachilidae) and the large clade of cleptoparasitic apids (Apidae; Cardinal et al. 2010), namely the subfamily Nomadinae, the tribe Melectini, and the taxa we refer to as the ericrocidine line (the tribes Rhathymini, Ericrocidini, Osirini, Protepeolini, Isepeolini, and the genus *Coelioxoides*). Although evidence suggests that both the apid genus *Exaerete* and the megachilid tribe Dioxyini may each exhibit two different cleptoparasitic strategies, insufficient information about their behavior, as well as a lack of phylogenetic representation from these groups in our data set, precluded their inclusion in MEDUSA analyses. We performed an independent diversification rate analysis on *Stelis* (*Stelis*) and the large clade of cleptoparasitic apids, in both cases collapsing terminals within each clade to more easily quantify the number of species represented by each terminal such that all extant taxa within the clade could be assigned to one (and only one) of these terminals. This left us with 12 terminal taxa in the *Stelis* clade and 18 terminal taxa in the apid clade. For each clade, we used MEDUSA to fit a series of 20 models of increasing complexity, where each successive model allowed for one more diversification rate shift than the previous model. We used a strict cut-off value of 10 as our ΔAIC threshold: when the AIC score for the best scoring model was less than 10 units less than the AIC score for the previous best-scoring model, the model search was stopped and the previous model retained.

To compare species richness among all lineages of cleptoparasites, we compared actual species diversity within cleptoparasitic clades to expected species diversity given a starting stem group age and underlying diversification rate (Magallón and Sanderson 2001). Average stem group ages of cleptoparasitic clades were taken from divergence dating analyses in BEAST. Clade species diversity (defined as the number of extant species within a clade) was taken from the relevant literature (Michener 2007; Table 1). We calculated the 95% confidence intervals of expected species diversity through time of a clade that diversifies with a rate equal to that of L-T bees as a whole in the absence of extinction ($e = 0.0$) and under a relatively high rate of extinction ($e = 0.9$) using equations (2b), (10a), and (10b) from Magallón and Sanderson (2001). The underlying rate of diversification for L-T bees was calculated using the *lambda.stem.ms01* function offered as part of the Laser package in R; this function calculates speciation and diversification rates given a known stem clade age and an underlying rate of extinction (Rabosky 2006; R-Development Core Team 2012).

To assess the impact of a cleptoparasitic life-history strategy on diversification rate, we compared the underlying diversification rate of each cleptoparasitic clade to that of the clade in which it was nested: diversification rates of cleptoparasitic subgenera

were compared to those of their respective genera, rates of genera were compared to those of their respective tribes, and rates of cleptoparasitic tribes were compared to those of their respective subfamilies. Diversification rate in the large clade of cleptoparasitic apids was compared to that of the family Apidae. We used *lambda.stem.ms01* with an extinction rate of $e = 0.9$ to calculate diversification rates. We assigned species numbers to each clade using Michener (2007) and used stem clade ages derived from our dating analysis in BEAST.

Results

ORIGINS OF CLEPTOPARASITISM IN MEGACHILIDAE

The results of phylogenetic analyses indicate six origins of cleptoparasitism in Megachilidae with no reversals to nest-building behavior (Figs. 1A, S1). Four cleptoparasitic clades are strongly supported as monophyletic and clearly represent independent origins of cleptoparasitism: (1) The anthidiine clade *Hoplostelis*+*Austrostelis* (100% posterior probability; 100% ML bootstrap support); (2) the anthidiine *Stelis* clade consisting of the genera *Stelis*, *Euaspis*, and *Afrostelis* (100% posterior probability; 100% ML bootstrap support; (3) the tribe Dioxyini (100% posterior probability; 100% ML bootstrap support); and (4) the *erythrogastra* species group of *Hoplitis* (*Hoplitis*); formerly the subgenus *Hoplitis* (*Bytinskia*); Sedivy et al. 2013a; 100% posterior probability; 100% ML bootstrap support).

Our results suggest one or two further origins of cleptoparasitism in the tribe Megachilini, the genus *Coelioxys* (100% posterior probability; 88% ML bootstrap support) and the genus *Radoszkowskiana* (represented in our phylogeny by a single species; Figs. 1A, S1). Relationships within the tribe are not well resolved and do not preclude the possibility that these two genera are in fact sister taxa. More detailed phylogenetic analyses will be required to determine the phylogenetic relationship between *Coelioxys* and *Radoszkowskiana*; ambiguity surrounding the relationship between these two taxa, however, does not affect our conclusions regarding the evolution of cleptoparasitic strategy in L-T bees.

EVOLUTION OF CLEPTOPARASITIC STRATEGY IN L-T BEES

Cleptoparasitic strategy is unknown for several lineages of L-T bees, including *Aglae* (Apidae, Euglossini), *Ctenoplectrina* (Apidae, Ctenoplectrini), most Dioxyini (Megachilidae), and the *erythrogastra* species group of *Hoplitis* (*Hoplitis*) (Megachilidae). The results of ML-based ancestral state reconstructions, however, strongly support the hypothesis that at least four of the 10 independent origins of cleptoparasitic behavior in L-T bees are associated with parasitizing closed nest cells. In Megachilidae, the common ancestor of the cleptoparasitic genera *Hoplostelis* and *Austrostelis* and the common ancestor of the *Stelis* group (*Stelis*, *Afrostelis*, and *Euaspis*; Fig. 1A) are both reconstructed as parasitizing closed nest cells with probabilities of 100% ($\Delta\ln L$ score between unconstrained analysis and analysis with ancestor of *Hoplostelis* and *Austrostelis* constrained to parasitizing open cells = 5.8; $\Delta\ln L$ score between unconstrained analysis and analysis with ancestor of *Stelis* clade constrained to parasitizing open cells = 6.4). In Apidae, the common ancestor of the large cleptoparasitic clade (the subfamily Nomadinae, the tribe Melectini, and the ericrocidine line) is reconstructed as parasitizing closed nest cells with a probability of 100% ($\Delta\ln L$ score between unconstrained analysis and analysis with ancestor of the large clade of cleptoparasitic apids constrained to parasitizing open cells = 4.3; Fig. 1A); the common ancestor of the genus *Exaerete* is also reconstructed as parasitizing closed nest cells with a probability of 100% ($\Delta\ln L$ score between unconstrained analysis and analysis with ancestor of *Exaerete* constrained to parasitizing open cells = 10.9).

While parasitizing closed nest cells is the plesiomorphic condition in both the *Stelis* clade and in the large clade of apid cleptoparasites, the common ancestors of both the subgenus *Stelis* (*Stelis*), nested within the *Stelis* clade (Megachilidae), and the subfamily Nomadinae, nested within the large apid clade, are reconstructed as parasitizing open nest cells with probabilities of 100% and 99%, respectively (Fig. 1A). The apid tribes Protepeolini (which includes only the genus *Leiopodus*) and Isepeolini (which includes only two genera, *Isepeolus* and *Melectoides*) and the osirine genus *Epeoloides* together form a monophyletic group derived from within the ericrocidine line (Figs. 1A, S1). Although the rest of the tribes in the ericrocidine line parasitize closed nest cells, the tribe Protepeolini exclusively parasitizes open nest cells; circumstantial evidence suggests that the genus *Epeoloides* and the tribe Isepeolini also parasitize open nest cells (Fig. 1A; Rozen 2003; Straka and Bogusch 2007).

Inferred transition rates between behavioral states based on maximum likelihood analyses are shown in Figure 1B. The rate of transition from parasitizing closed cells to parasitizing open cells (0.0043) is more than two and a half times higher than the rate of transition from nest-building to parasitizing closed cells (0.0016) and almost 15 times higher than the rate of transition from nest-building to parasitizing open cells (0.00029). Analyses where the rate of transition from nest-building to parasitizing open nest cells was constrained to 0 resulted in a likelihood score 1.5 likelihood units lower than the likelihood score of the unconstrained analysis, indicating that this rate is not significantly different from 0. The rates of transition from parasitizing both open and closed cells to nest-building are 0. The rate from parasitizing open cells to parasitizing closed cells is also 0.

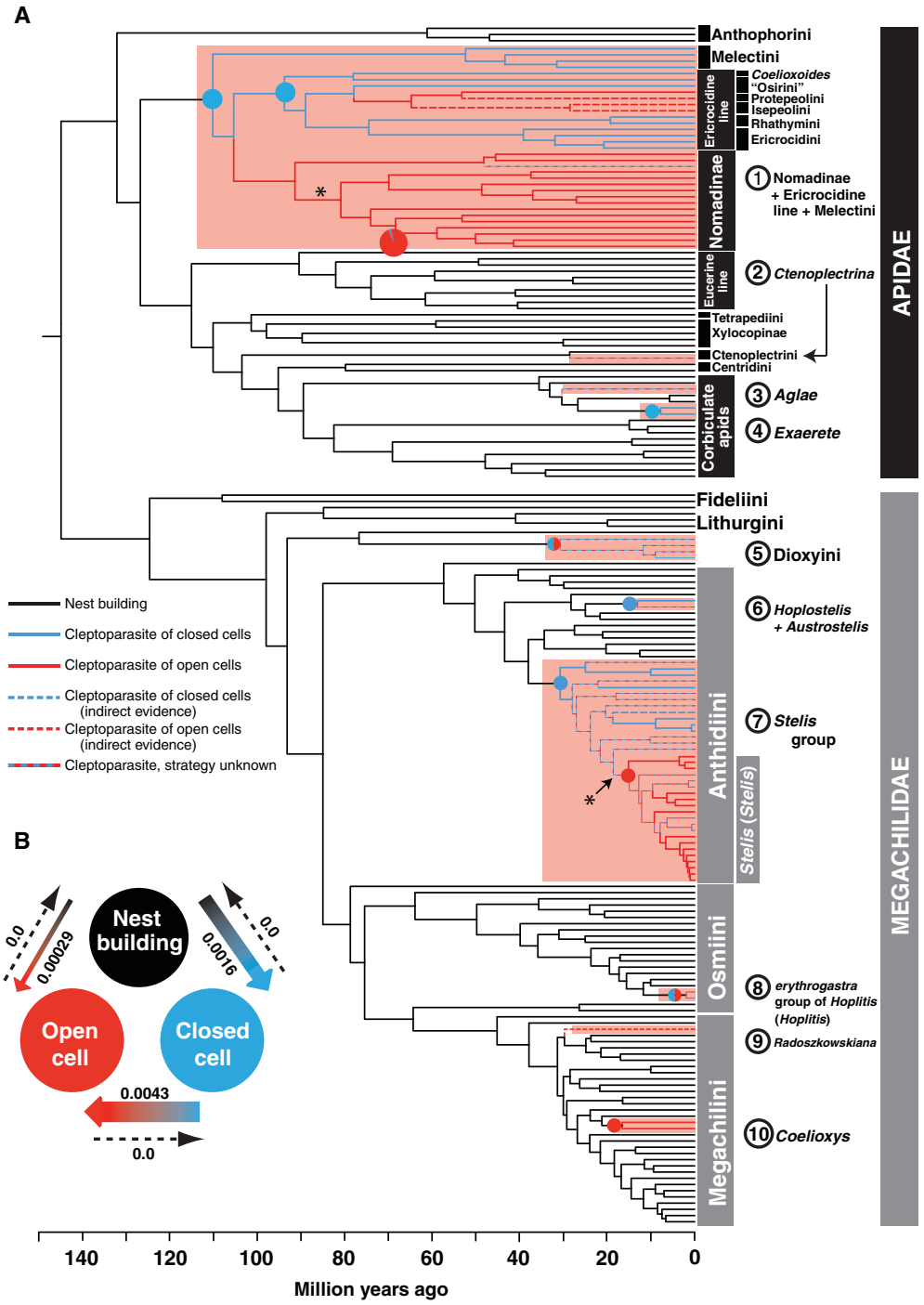

Figure 1. (A) Maximum clade credibility tree of long-tongued (L-T) bees obtained from BEAST analysis. Cleptoparasitic clades are highlighted in pink. Names of cleptoparasitic clades are numbered one through ten to the right of the phylogeny. Ancestral state reconstructions are represented as pie charts at ten selected nodes. For both ancestral state reconstructions and branches, black = nest-building, blue = cleptoparasites of closed nest cells, red = cleptoparasites of open nest cells, broken blue = cleptoparasites of closed nest cells based on indirect evidence, broken red = cleptoparasites of open nest cells based on indirect evidence, blue-red = cleptoparasites of unknown strategy. Branches are colored according to the criterion of maximum parsimony. Asterisks at the base of *Stelis* (*Stelis*) and Nomadinae indicate nodes exhibiting a shift in diversification rate as determined by MEDUSA analyses. Clades within subgenus *Stelis* (*Stelis*) are colored red based on known behavior of other species in the same species group. Phylogenetic placement of apid tribe Caenoprosopidini (represented by blue-red line in Nomadinae), not included in our analysis, is based on Cardinal et al. (2010), figure 1. Outgroup taxa have been pruned out for ease of presentation. (B) Rates of transition between behavioral states obtained from ancestral state reconstructions in BayesTraits. Black circle represents nest building behavior, blue circle indicates cleptoparasites of closed nest cells, and red circle indicates cleptoparasites of open nest cells. Rates represent changes between states per million years.

DIVERSIFICATION RATES AND CLADE SPECIES RICHNESS

A cleptoparasitic life-history strategy is associated with a decrease in diversification rate in eight of 10 cleptoparasitic clades (Table 1). Only the *Stelis* clade and *Coelioxys* exhibited a clade-wide increase in diversification. The results of MEDUSA analyses performed on the *Stelis* clade and the large clade of cleptoparasitic apids indicate that the transition to parasitizing open nest cells coincides with a significant increase in diversification rate. In the *Stelis* clade, we find an increase in diversification rate from 0.0797 to 0.130 that occurs at the base of the subgenus *Stelis* (*Stelis*) and which coincides with the transition to parasitizing open nest cells seen in that subgenus (Fig. 1A). We also find a significant increase in diversification rate from 0.0444 to 0.0726 in the large cleptoparasitic apid clade; this increase in diversification rate occurs approximately 10.7 million years after the transition to parasitizing open nest cells which, in this clade, is first seen in the tribe Ammobatini (Figs. 1A, S1). However, the other transition to parasitizing open nest cells in this clade, in the ancestor of Isepeolini, Protepeolini and the genus *Epeoloides*, was not associated with a significant change in diversification rate.

Our results also support an association between clade species richness and parasitizing open cells. According to the criterion set forth by Magallón and Sanderson (2001), clades that fall above the upper limit of the highest confidence interval of expected species diversity ($e = 0.9$) are considered extremely species rich; the results of our tests for clade species diversity across all cleptoparasitic clades in the L-T bees indicate that two clades fall into this category: *Stelis* (*Stelis*) and *Coelioxys* (Fig. 2), both of which parasitize open cells. Although the phylogenetic position of *Coelioxys* is not well-resolved in our phylogeny, it is inarguably nested within the clade containing the genus *Megachile*. The crown clade age of the genus *Megachile* is 38.5 million years; even assuming this age for *Coelioxys* places it above the upper limit of the highest confidence interval of expected species diversity. The subfamily Nomadinae falls just below the lower limit of the highest confidence interval. Clades that fall below the lower limit of the lowest confidence interval of expected species diversity ($e = 0.0$), however, are considered extremely species poor. Three clades fall into this category: *Stelis* (*Protostelis*), *Aglae*, and *Coelioxoides*+*Parepeolus* (Fig. 2).

Discussion

ORIGINS OF CLEPTOPARASITISM AND EVOLUTION OF CLEPTOPARASITIC STRATEGY

Michener (2007) proposed 10 independent origins of cleptoparasitism within Megachilidae, six of those in the tribe Anthidiini. Our results, however, strongly support two independent origins in Anthidiini. The results of our molecular phylogenetic analyses thus imply that the number of origins of cleptoparasitism in Megachilidae is six (or five, if *Coelioxys* and *Radoszkowskiana* form a monophyletic group); taken together with the results of Cardinal et al. (2010), the total number of independent origins of cleptoparasitism in the L-T bees is nine or 10.

The results from our ancestral state reconstructions suggest that cleptoparasitic behavior evolves unidirectionally, from parasitizing closed nest cells to parasitizing open nest cells with no evidence of reversals. Although it has been suggested that the behavioral and morphological adaptations seen in hospicidal larvae represent derived character states (Rozen 1991), our results are the first to indicate an evolutionary transition from parasitizing closed nest cells to parasitizing open nest cells. We thus propose the following evolutionary pathway to describe the evolution of cleptoparasitic behavior in bees.

Phase 1: Nest building phase

The majority of solitary bees are nest-building. Under certain conditions, however, it may be more advantageous for a female bee to parasitize the nest of another bee rather than to build and provision her own nest (Yamane 1978; Matsuura and Yamane 1984; Wcislo 1987). Cleptoparasitism may first arise intraspecifically as a facultative behavior, in which a female usurps the nests of conspecific females in addition to building her own nests (Eickwort 1975; Iwata 1976; Wcislo 1987; Field 1992; Cichon 1996). The evolution of an obligately cleptoparasitic lineage, however, most likely depends on a switch to a host belonging to a different species; indeed, the sympatric split of a host–parasite species pair from the same population of bees is unlikely (Eickwort 1975), although such scenarios have been suggested in socially parasitic ants (reviewed in Buschinger 2009).

Phase 2: Closed nest cell parasitized, adult female cleptoparasite kills host offspring (AC strategy)

Our results from BayesTraits indicate a 5.5-fold higher transition rate between nest-building and parasitizing closed nest cells than between nest-building and parasitizing open cells. Moreover, our results also suggest that the rate of transition from nest-building to parasitizing open nest cells is not significantly different from 0. Taken together, these results indicate that every new origin of cleptoparasitism involves a preliminary phase in which the adult female cleptoparasite usurps a closed nest cell. This hypothesis is supported by the fact that facultative, intraspecific brood parasitism in bees, and apoid wasps mostly involves the opening of closed cells and the destruction and replacement of the host egg by the usurping female (Eickwort 1975, and citations therein; Field 1992). As further evidence, solitary bee larvae are not aggressive, nor are they equipped to kill other larvae. Consequently, the larvae of a newly derived cleptoparasitic lineage would not yet have evolved the behavior or morphology necessary to kill the

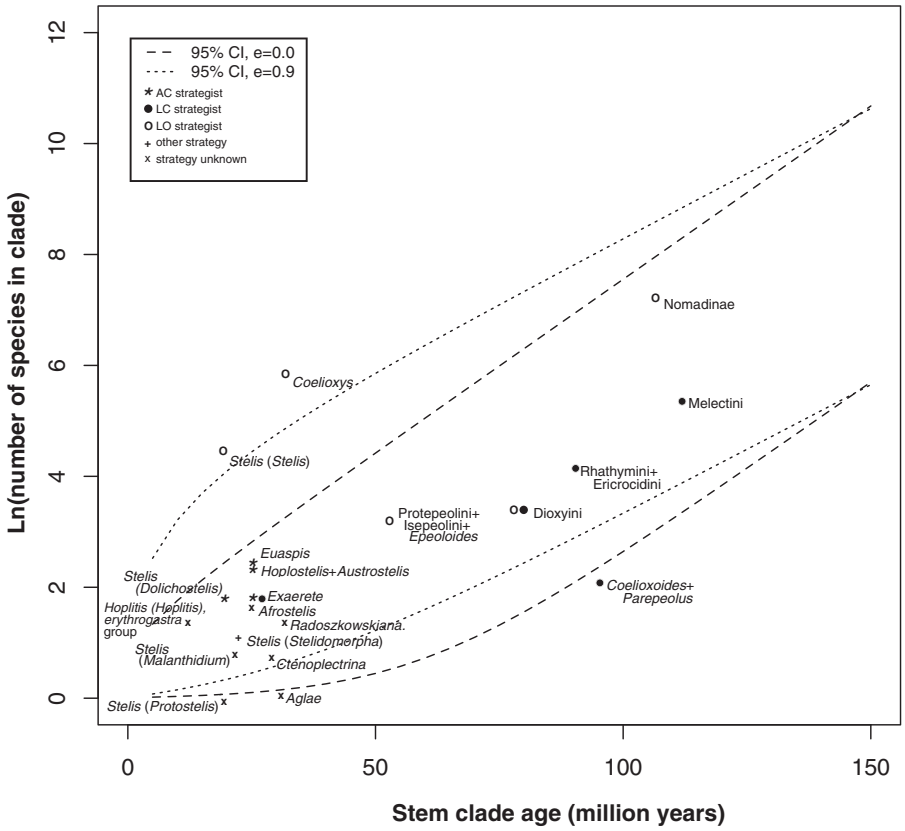

Figure 2. **Cleptoparasitic clades plotted according to stem clade age and species number per clade. Confidence intervals of expected species diversity are given as a function of stem clade age. The 95% confidence intervals are based on expected species diversity through time of a clade that diversifies with a rate equal to that of long-tongued (L-T) bees as a whole in the absence of extinction ($e = 0.0$, $rate_{0.0} = 0.06248$, dashed line) and under a relatively high rate of extinction ($e = 0.9$, $rate_{0.9} = 0.04682$, dotted line). AC strategists are marked by an asterisk; LC strategists are marked with a closed circle; LO strategists are marked with an open circle; cleptoparasites of unknown strategy are marked with an "x." Members of the genus *Exaerete* may be either AC or LC strategists; this genus is marked with both an asterisk and a closed circle. Members of the tribe Dioxyini are parasites of both closed and open cells; this tribe is marked with both a closed and an open circle. See caption to Table 1 for details regarding the behavior of *Stelis* (*Stelidomorpha*), here marked by a "+." The cleptoparasitic strategy of a number of clades shown here is unknown. Based on the ages of these clades, clade size, the unmodified abdominal apex of females, and the close phylogenetic relationship with their hosts (where known), we predict that *Stelis* (*Protostelis*), *Stelis* (*Malanthidium*), *Aglae*, the *erythrogastra* species group of *Hoplitis* (*Hoplitis*), and *Afrostelis* are AC strategists.**

offspring of its host, thus the destruction of the host's offspring would have to be carried out by the adult cleptoparasitic female.

Phase 3: Closed nest cell parasitized, cleptoparasitic larva kills host offspring (LC strategy)

A cleptoparasitic strategy in which the cleptoparasitic larva kills the host's offspring represents a reduction in the amount of time required to parasitize a single cell: the adult female cleptoparasite makes a small opening in the cell, deposits her egg, and then re-seals the opening; no time is spent removing the host's offspring or reworking the pollen mass and the small hole required for oviposition is more rapidly made and more rapidly closed than the larger one she is required to make as an AC strategist. The evolution of hospicidal larvae has occurred at least five times independently in bees and represents a major innovation that ensures the destruction of the host's offspring and allows the cleptoparasitic female time to parasitize more cells. Furthermore, the evolution of hospicidal larvae may have been driven not only by a need to eliminate the host but also other cleptoparasites present in the same cell (Rozen et al. 2006). Indeed, nest cells that are parasitized once are likely to be parasitized again (Field 1992 and references therein), placing the offspring of the cleptoparasite in competition not only with the offspring of the host but also with other cleptoparasites deposited in the same nest cell.

Phase 4: Open nest cell parasitized, cleptoparasitic larva kills host offspring (LO strategy)

The LO strategy allows nest cells to be parasitized before the host larva has begun to consume the pollen mass, thus ensuring that cleptoparasitic larvae have the entire pollen contents of a

nest cell at their disposal. Parasitizing open cells may also give cleptoparasitic offspring a developmental edge over any other cleptoparasites deposited in the same cell: the first hospicidal larva deposited in a cell is likely to be the first to emerge and thus the first to kill other competitors. Finally, open nest cells represent a potentially vast resource that is inaccessible to parasites restricted to closed nest cells. The LO strategy has evolved independently at least five times in bees and is the most prevalent strategy within the group (in terms of species numbers); furthermore, there is no phylogenetic evidence of reversals from this strategy to any other, implying an evolutionary advantage associated with parasitizing open nest cells.

UNDERSTANDING FAMILY-SPECIFIC ORIGINS OF CLEPTOPARASITISM

In 1871, Hermann Müller observed that the hosts of cleptoparasitic bees are often other species of bees to which they are closely related (Müller 1871); nearly four decades later, Carlo Emery made a similar observation regarding the appearance of social parasitism in ants (Emery 1909). This trend, originally called "Müller's Law" (Popov 1945), also often referred to as "Emery's Rule," is particularly apparent in young cleptoparasitic clades: the *erythrogastra* species group of *Hoplitis* (*Hoplitis*) on closely related species belonging to the same subgenus (Megachilidae, Osmiini; Sedivy et al. 2013b); *Coelioxys* and *Radoszkowskiana* on *Megachile* (Megachilidae, Megachilini; Rozen et al. 2010 and references therein); *Exaerete* and *Aglae* on *Eufriesea* and *Eulaema* (Apidae, Euglossini; Bennett 1972; Garófalo and Rozen 2001); *Austrostelis* on *Epanthidium* (Megachilidae, Anthidiini; Zanella and Ferreira 2005); *Dialictus* on other species of *Dialictus* (Halictidae, Halictini; Michener 1978), and likely *Ctenoplectrina* on *Ctenoplectra* (Apidae, Ctenoplectrini; Schaefer and Renner 2008).

The host preferences of basal members of older cleptoparasitic clades also provide evidence that Müller's observation applies to older clades: basal members of the large clade of apid cleptoparasites attack other apids, namely Anthophorini (Rozen 1991 and references therein); Dioxyini, a megachilid tribe whose phylogenetic affinities are unclear (Litman et al. 2011), restricts its host range to other megachilids (Rozen and Özbek 2005); basal members of the *Stelis*-clade attack resin-nesting megachilids, including other Anthidiini (Michener 1955; Iwata 1976; Parker et al. 1987; Westrich 1989); and the halictine genus *Sphecodes* is closely associated with other Halictini (Michener 1978).

Eickwort (1975) formulated a likely explanation for the phylogenetic relatedness between cleptoparasite and host: a female cleptoparasite must be able to locate an appropriate host nest using some combination of visual and olfactory cues. Nests most likely to be recognized as "appropriate" by a newly derived cleptoparasite are therefore ones which closely resemble nests made by the nest-building ancestor of the parasite and which may still be made by closely related species (Eickwort 1975). This observation, together with the pathway presented above, may help explain the conspicuous discrepancy concerning the appearance of cleptoparasitic lineages among bee families (Wcislo 1987). Our results, together with those of Cardinal et al. (2010), indicate nine or 10 independent origins of cleptoparasitism in the bee families Megachilidae and Apidae; nine additional origins are posited in the subfamily Halictinae, although some of these origins may in fact represent social parasites and not cleptoparasites (Michener 2007; Gibbs et al. 2012). Yet there are no confirmed cleptoparasitic lineages in the families Andrenidae, Colletidae, and Melittidae, nor in the halictid subfamilies Nomiinae and Rophitinae (although see Daly and Magnacca [2003] regarding purported cleptoparasitism in the colletid subgenus *Hylaeus* [*Nesoprosopis*]). If newly derived cleptoparasitic lineages parasitize closed nest cells of closely related bees, then the architecture of nests built by members of the families Andrenidae, Colletidae, Melittidae, and the halictid subfamilies Nomiinae and Rophitinae may have greatly impeded the evolution of cleptoparasitism in these families.

Bees belonging to these lineages often build branching nests in the ground, in which single nest cells are located at the ends of tunnels that are backfilled with excavated substrate. Such nest cells would be difficult to find for newly derived, thus AC, cleptoparasites, given that a tunnel filled with sand leaves little evidence of its location; such cells, however, may be readily found by LO strategists. AC strategists in lineages such as the megachilid genera *Hoplostelis*, *Stelis* (*Dolichostelis*), and *Euaspis*; the apid genus *Exaerete*; and most members of the halictid genus *Sphecodes*, parasitize hosts whose nest cells are either arranged in compact clusters or linearly in open cavities, such as the nests of *Euglossa*, *Megachile* (*Callomegachile*), and *Megachile* (*Chelostomoides*), or whose nests contain many cells located in close proximity to a main nest burrow, such as the nests of most *Lasioglossum*. Such nests may be easier for AC strategists to parasitize and may explain the multiple origins of cleptoparasitism seen in Megachilidae, Apidae, and Halictinae. Furthermore, facultative intra-specific nest usurpation, one of the first manifestations of cleptoparasitic behavior, has been reported in members of the families Megachilidae, Apidae, and Halictidae (Wcislo 1987; Field 1992) but is almost unheard of in other bee families (although see Rozen [1994] for a possible case of intraspecific parasitism in the colletid genus *Ptiloglossa*), providing further support to the argument that cleptoparasitic lineages may arise more readily in these families than in others.

Remarkably, some cleptoparasites of closed cells, such as *Melecta separata callura* (Apidae, Melectini) on *Anthophora edwardsii* (Apidae, Anthophorini; Thorp 1969) and *Sphecodes albilabris* (Halictidae, Halictini) on *Colletes cunicularius* (Colletidae,

Colletini) (Westrich 1989) have evolved a means to locate and parasitize closed host nests that are buried at the ends of tunnels in the soil, possibly in response to olfactory cues coming from the nesting material itself, such as Dufour's gland secretions.

PATHWAY BEYOND BEES

Other lineages of cleptoparasitic Aculeata exhibit strategies consistent with those seen in bees. Members of the wasp family Pompilidae prey on spiders. Two clades, however, the genus *Evagetes* and subfamily Ceropalinae, are entirely cleptoparasitic. Female *Evagetes* enter the closed nest cells of other pompilids, destroy the host's egg, and deposit their own egg on the prey stored by the host (Evans 1953). Some members of Ceropalinae, however, deposit their eggs in prey caught by other pompilids *before* the prey is brought into the nest; others, such as *Irenangelus eberhardi*, deposit their eggs in open nest cells that have already been provisioned (Wcislo et al. 1988). Once in the nest, the cleptoparasitic ceropaline larva emerges before that of the host and destroys the host's offspring (Evans 1953). Two further clades of obligate brood parasites are found in the apoid wasp family Crabronidae, the genus *Stizoides* and the tribe Nyssonini. *Stizoides* burrows into the closed nests of other apoid wasps, destroys any offspring present, and deposits its egg on the provisions inside the nest cell (O'Neill 2001). In contrast, female members of the tribe Nyssonini hide their eggs on their host's provisions before the host's egg has been laid; the cleptoparasitic larva emerges first, kills the host's offspring, and develops on the provisions (O'Neill 2001). The strategies of both *Evagetes* and *Stizoides* mirror those of AC strategists, whereas Ceropalinae and Nyssonini clearly display an LO strategy.

Evans (1953) hypothesized that the behavior of cleptoparasitic pompilids was a derivation of the behavior seen in nest-building members of the family. He suggested that the type of cleptoparasitic behavior exhibited by *Evagetes* (by our definition an AC strategist) was an intermediate step between nest-building and the type of behavior exhibited by Ceropalinae (by our definition an LO strategist) and concluded that "... at one time in its evolution, *Ceropales* [Ceropalinae] probably behaved very much as *Evagetes* does now" (Evans 1953). If individual genera are likely to be younger than both subfamilies and tribes, then younger clades (e.g., *Evagetes* and *Stizoides*) exhibit behavior consistent with AC strategists, whereas older clades (e.g., Ceropalinae and Nyssonini) exhibit derived behavior that is consistent with LO strategists in bees. This, in conjunction with the conclusions of Evans (1953), suggest that the pathway that we propose, which includes an early evolutionary phase as an AC strategist and a derived phase as an LO strategist, may be extended beyond bees to describe the evolution of cleptoparasitic behavior in other clades of Aculeata.

Exceptions to this pathway exist, however. The cleptoparasitic wasp family Sapygidae, all members of which exhibit a strategy similar to the LO strategy seen in bees, is likely the sister group to Mutillidae (Heraty et al. 2011), a group of ectoparasitoids on the superfamily Apoidea. It is tempting to conclude that sapygids are derived from ectoparasitoid ancestors whose larvae were already equipped for killing their hosts, in contrast to the larvae of the nest-building ancestors of cleptoparasitic apoid and pompilid wasps. Similarly, true cleptoparasitic lineages are an exception in the cuckoo wasps (Chrysididae), most of which are ectoparasitoids on the mature larvae or pupae of apoid wasps. Thus in Sapygidae and Chrysididae, brood parasitism may have evolved via a distinct pathway from that seen in bees, apoid wasps, and spider wasps, that is not directly from nest-building ancestors but rather from ectoparasitoid ancestors with larvae already equipped to destroy their hosts.

DIVERSIFICATION IN CLEPTOPARASITIC LINEAGES

We find evidence for an overall decrease in diversification rate in eight of 10 cleptoparasitic bee clades compared to the clades they are nested within (Table 1). Recent studies have shown that this trend is echoed in birds, where brood parasitic cuckoo clades exhibit lower rates of diversification compared to their relatives with parental care; these decreases in diversification are driven by increased rates of extinction in brood parasitic clades (Krüger et al. 2009). Not only is the success of a cleptoparasite inextricably tied to that of its host, parasites must likely exist at relatively low numbers to maintain density-dependent host–parasite equilibrium. Thus, the relatively low population densities that characterize cleptoparasitic populations, combined with a specialization on a limited number of hosts (Dunn et al. 2009), likely place cleptoparasitic clades at higher risk of extinction and may explain the lower diversification rates seen in these clades. Although our estimates of diversification rate are based on calculations using a fixed extinction rate ($e = 0.9$), we believe that the decrease in diversification rate that we observe in most cleptoparasitic clades is at least partially due to an increased rate of extinction (which is not discernible from a decreased rate of speciation in our analyses).

Two clades, however, exhibit an overall increase in diversification rate compared to the clades they are nested in: the *Stelis* clade and *Coelioxys* (Table 1). These two groups, both LO strategists, also fall above the upper limit of the highest confidence interval of expected species diversity ($e = 0.9$; Fig. 2). AC strategists, however, are both relatively young (all less than approximately 30 million years old) and relatively species poor (all containing 12 species or less; Fig. 2).

MEDUSA analyses reveal that the transition from closed to open cell parasitism is associated with an increase in the rate of diversification in both the large clade of cleptoparasitic apids

(associated with a shift from LC to LO) and in the *Stelis* clade (associated with a shift from AC to LO). Basal members of the apid clade are mostly restricted to parasitizing other apids, whereas lineages belonging to the subfamily Nomadinae and the tribes Protepeolini (and probably Isepeolini), all LO strategists, typically parasitize hosts belonging to diverse genera of the families Andrenidae, Halictidae, Colletidae, and Melittidae.

Host associations in the *Stelis* clade are less known and conclusions are more difficult to draw. Limited observations suggest, however, that basal members of the *Stelis* clade, all known members of which are AC strategists, are host restricted (Table 1). Although these hosts span several tribes of megachilids, their nests are built almost exclusively of plant resin. The *Stelis* clade is derived from within the clade of resin-nesting anthidiines, leading us to hypothesize that AC strategists in the *Stelis* clade may require resin as a cue to locate the closed nest cells of their hosts. In contrast, host association in the subgenus *Stelis* (*Stelis*), all LO strategists, appears more dynamic. Some European species are notably generalist in their choice of hosts (e.g., *Stelis punctulatissima* on diverse anthidiines, megachilines, and osmiines). Moreover, closely related species may parasitize widely divergent hosts exhibiting a rich array of nesting behavior (e.g., *Stelis simillima*, closely related to *S. punctulatissima*, is a parasite of *Lithurgus*).

The transition to LO strategy seen in the *Stelis* clade and in the large clade of cleptoparasitic apids likely relaxed the constraints associated with parasitizing closed cells and facilitated host-switches to as-of-yet unparasitized lineages that may have been inaccessible to AC or LC cleptoparasites due to the architecture of their nests. We see host clade size, and not cleptoparasitic strategy itself, as the primary factor driving the diversity seen in LO clades. Species richness in nymphalid butterflies is strongly correlated with diversity of host use (Janz et al. 2006) and diversification in agromyzid flies is associated with shifts to new host plant clades (Winkler et al. 2009). Host-switches may also have expanded the geographical range of cleptoparasites, correlated with diversification in other groups, including land snails, flowering plants, and cichlid fish (Parent et al. 2006; Seehausen 2006; Vamosi and Vamosi 2010).

Conclusion

Diverse lineages of brood parasites, including birds and bees, display decreased rates of diversification, indicating a trade-off between the immediate ease of stealing the nests of other organisms (Field 1992 and references therein) and the long-term evolutionary risks associated with obligate cleptoparasitism. Despite these risks, brood parasitism has evolved multiple times independently in aculeate Hymenoptera; in at least six of these lineages, the evolution of such behavior has culminated in a strategy in which open nest cells are parasitized. The transition to such a strategy is characterized by a number of remarkable evolutionary parallelisms, including hospicidal offspring that emerge before those of their hosts and eggs with unique sizes, patterns, and textures to facilitate egg-hiding in host nests. Combinations of these convergences also appear in other brood parasites including catfish (Sato 1986) and three independent lineages of avian brood parasites (Spottiswoode and Koorevaar 2012), indicating the fierce competition for resources between host and brood parasite and hinting at evolutionary pressure to eliminate competitors for these resources.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Maximum clade credibility tree of long-tongued (L-T) bees obtained from BEAST analysis.

Table S1. Collection localities and DNA voucher numbers for all new taxa included in this study.

Table S2. GenBank accession numbers for all taxa included in this study.