The evolution and loss of oil-offering flowers: new insights from dated phylogenies for angiosperms and bees

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The interactions between bees that depend on floral oil for their larvae and flowers that offer oil involve an intricate mix of obligate and facultative mutualisms. Using recent phylogenies, new data on oil-offering Cucurbitaceae, and molecular-dating, we ask when and how often oil-offering flowers and oil-foraging bees evolved, and how frequently these traits were lost in the cause of evolution. Local phylogenies and an angiosperm-wide tree show that oil flowers evolved at least 28 times and that floral oil was lost at least 36–40 times. The oldest oil flower systems evolved shortly after the K/T boundary independently in American Malpighiaceae, tropical African Cucurbitaceae and Laurasian *Lysimachia* (Myrsinaceae); the ages of the South African oil flower/oil bee systems are less clear. Youngest oil flower clades include *Calceolaria* (Calceolariaceae), Iridaceae, *Krameria* (Krameriaceae) and numerous Orchidaceae, many just a few million years old. In bees, oil foraging evolved minimally seven times and dates back to at least 56 Ma (*Ctenoplectra*) and 53 Ma (*Macropis*). The co-occurrence of older and younger oil-offering clades in three of the four geographical regions (but not the Holarctic) implies that oil-foraging bees acquired additional oil hosts over evolutionary time. Such niche-broadening probably started with exploratory visits to flowers resembling oil hosts in scent or colour, as suggested by several cases of Muellierian or Batesian mimicry involving oil flowers.

Keywords: oil-offering flowers; oil-foraging bees; molecular clock dating; evolutionary gain; evolutionary loss; oil biochemistry

1. INTRODUCTION

The single most striking change in the study of plant/animal interactions over the past 15 years has been the increasing role of phylogenies and the comparative approach in the study of mutual adaptation and coevolution. Work on flower/pollinator interactions, especially, has benefited from the ability of molecular phylogenies to shed light on the relative and absolute sequence of the evolution of traits and on the degree of temporal or geographic congruence in the evolutionary histories of interacting partners. Most flower/pollinator mutualisms are facultative (Bronstein 1994; Thompson 1999). Obligate mutualisms between plant species and their pollinators by contrast are rare. Nevertheless, they offer the opportunity to examine reciprocal adaptation in detail and have become models for understanding how mutualisms sometimes lead to coevolution (e.g. Bogler *et al.* 1995; Pellmyr *et al.* 1996; Pellmyr & Leebens-Mack 2000; Whittall & Hodges 2007). Here we focus on a bee/flower mutualism that comprises a fascinating mix of obligate and facultative dependencies, with numerous reciprocal

morphological, behavioural and chemical adaptations. This is the mutualism between oil-offering flowers and bees that depend on floral oil for larval provisioning and cell lining, a mutualism discovered 40 years ago (Vogel 1969, 1974, 1981a,b, 1984, 1990).

Oil flower/oil bee mutualisms involve highly specialized bees belonging to a few genera in the Melittidae and Apidae (including the former Anthophoridae and Ctenoplectridae; Michener 2007). Some of these bees use floral lipids with, or in place of nectar to be mixed with pollen for larval provisioning. Others use the oils not only as larval foodstuffs but also for water-resistant cell linings (Cane *et al.* 1983; Vogel 1988; Alves dos Santos *et al.* 2002; Melo & Gaglianone 2005). Earlier reviews of the oil flower syndrome (Neff & Simpson 1981; Buchmann 1987; Rasmussen & Olesen 2000; Machado 2004) provide excellent summaries of the geographic distribution and adaptations of the relevant flowers and their bees. Machado (2004) also includes species-level lists of interacting partners. New oil bee/oil flower interactions continue to come to light (Manning & Goldblatt 2002; H. Schaefer 2008, unpublished observations for African and Asian Cucurbitaceae), especially in tropical Orchidaceae, where they are scattered widely and floral biology is poorly known (Reis *et al.* 2006; Singer *et al.* 2006; Stępczyńska *et al.* 2007; Pansarin *et al.* 2008, 2009). Some plants also produce oil-rich and semi-liquid pollenkitt, for example, the Zingiberaceae *Caulokaempferia coenobialis* (Wang

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et al. 2004, 2005) and a few species of the Myrtaceae *Verticordia* (Houston *et al.* 1993). We consider such pollen a reward distinct from the floral oils that are the focus of this paper because it is produced by the inner anther wall (the tapetum), not outer epithelia or trichomes, and is taken up with the glossae, not hairs or brushes on legs and abdomens (as are floral oils).

None of the earlier reviews of oil flower/oil bee mutualisms were able to take advantage of large phylogenies and dated chronograms for the relevant bees and plant clades. Yet, date phylogenies can help answer the question to what extent oil offering in flowers and oil foraging in bees evolved at similar geological times and hence how much evolutionary host broadening or switching may have occurred between pre-existing plant or pollinator clades. Also, when Buchmann (1987) wrote his review of the ecology of oil flowers and bees, no reliable fossils were known of any oil bee. This has changed with the description of an oil-collecting bee from the Eocene (53 Myr) of France (Michez *et al.* 2007), and there is also an Oligocene (34 Myr) oil gland-bearing malpigh flower from Tennessee (Taylor & Crepet 1987; confirmation of phylogenetic assignment in Davis *et al.* 2004).

Based on published phylogenies, our own work on oil-offering Cucurbitaceae and their bees (Schaefer & Renner 2008, 2009), and sequence matrices assembled for this study to carry out molecular dating, we here address the following questions: (i) how often and during which geological times did oil-offering angiosperm clades evolve; (ii) at which times did the relevant oil-foraging bees originate; (iii) how often and under which conditions were oil offering and oil foraging lost? The answers to these questions then form the basis for a discussion of likely evolutionary pressures underlying the gain and loss of oil rewards.

2. METHODS

(a) *Phylogenetics of oil-producing angiosperms and oil-foraging bees*

We tabulated all known oil-offering angiosperms with their family placement according to the system proposed by the Angiosperm Phylogeny Group (2003), geographic occurrence, associated oil bees and key references on the interaction. To infer global relationships and genetic distances from close relatives, we downloaded 624 *rbcL* sequences from GenBank. For *Nierembergia* and *Monttea*, we produced new *rbcL* sequences (GenBank accession numbers FJ911661–FJ911662). We similarly tabulated all known oil-foraging bees, with their phylogenetic placement (Michener 2007; Schaefer & Renner 2008; Michez *et al.* 2009b; S. Cardinal 2009, Cornell University, personal communication), documented or inferred geological age, and relevant references.

(b) *DNA data generation, phylogeny estimation and evolutionary rate analyses*

Techniques for DNA extraction, sequencing and alignment follow Schaefer *et al.* (2009). Maximum-

likelihood (ML) tree searches and ML bootstrap searches were performed using RAxML v. 7.0.4 (Stamatakis *et al.* 2008; available at <http://phylo.bench.vital-it.ch/raxml-bb/>). RAxML searches relied on the GTR + G model (six general time-reversible substitution rates, assuming gamma rate heterogeneity, with 25 gamma rate categories), and model parameters were estimated over the duration of specified runs.

For divergence time estimates, sequence subsets for asterids, Ericales, rosids and Lamiales were compiled from GenBank and the sequences aligned by eye using MacClade v. 4.08 (Maddison & Maddison 2003). Bayesian uncorrelated-rates clock estimations used the approach of Drummond *et al.* (2006), with alignments first imported in BEAUti v. 1.4.8 (part of the BEAST package; Drummond & Rambaut 2007) to generate BEAST input files. In BEAST, we used the GTR + G model with six gamma rate categories. The ages of the following most recent common ancestors were constrained based on fossils or in agreement with Wikström *et al.* (2001), all with normal prior distributions: The Ericales stem to 110 ± 4 Myr, the Zygophyllales stem to 98 ± 3 Myr, the Fagales stem to 84 Myr, the *Eucommia* stem to 59–55 Myr, the Icacinaceae crown to 70 ± 5 Myr, the Stilbaceae stem to 49 ± 3 Myr and the *Plantago-Antirrhinum* split to 48 ± 3 Myr. Eucommiaceae fossils are first known in the Late Cretaceous of Europe, but are most common in Eocene to Oligocene sediments (Call & Dilcher 1997); the oldest fruits of Icacinaceae (tribe Iodeae) come from the Late Paleocene of western North America (Pigg *et al.* 2008). The earliest Fagales fossils may be *ca* 84 Myr old (Herendeen *et al.* 1995), although the *Normapolles* genus *Caryanthus*, which belongs to crown group Fagales, is known from the Cenomanian and onwards (Friis *et al.* 2006). The performance of the BEAST runs was checked using Tracer v. 1.4 (Rambaut & Drummond 2007). The resulting trees were combined using TreeAnnotator v. 1.4.8 (part of the BEAST package), with a burnin of 1000 trees. Final trees were checked and edited in FigTree v. 1.2 (Rambaut 2006–2008).

3. RESULTS

(a) *Number of independent gains and losses of floral oil*

Table 1 shows 28 independent origins of oil-offering flowers indicated by current phylogenies. An angiosperm-wide *rbcL* phylogeny (figure 1) provides a visual overview of the distribution of oil as a reward in flowering plants. Oil-offering flowers are known from 11 families that occur mainly in the tropics and subtropics. In most of these families oil glands evolved only once, but in Iridaceae and Orchidaceae oil offering evolved multiple times (Goldblatt *et al.* 2008; Chase *et al.* 2009; M. Whitten 2009, personal communication; our table 1 and figure 1). For the latter family, phylogenetic understanding is too incomplete to infer the numbers of gains (or losses) in detail. It is safe to assume, however, that orchids harbour many more than the 12 independent origins of oil flowers listed in table 1.

Table 1. The 28 independent origins of oil-offering flowers indicated by current phylogenies. An angiosperm-wide *rbcL* phylogeny (figure 1) provides a visual overview of the distribution of oil as a reward in flowering plants.

independent origins of oil-offering flowers; genera with species numbers and geographical range	age of clade (stem or crown as indicated) ^a	characteristic oil-foraging bees	selected references on: pollination interaction/plant clade age
<i>America</i>			
Calceolariaceae: <i>Calceolaria</i> (260, Andes to Chile), at least 49 of <i>ca</i> 260 species lack oil	<i>Jovellana</i> / <i>Calceolaria</i> split: 19 Myr ¹ ; 15 (27–4) Myr ² <i>Calceolaria</i> crown group: 5 (6–1) Myr ²	<i>Centris</i> , <i>Chalepogenus</i>	Vogel (1974) and S�rsic (2004). Phylogeny see Andersson (2006)/ ¹ Datson <i>et al.</i> (2008), ² this study
Iridaceae I: <i>Sisyrinchium</i> (80–110, N. and S. America), only the 35 S. American species oil-offering	stem lineage: <i>ca</i> 22 Myr	<i>Chalepogenus</i> , <i>Lanthanomelissa</i>	Vogel (1974), Roig-Alsina (1997) and Cocucci & Vogel (2001)/Goldblatt <i>et al.</i> (2008)
Iridaceae II: Tigridieae + Trimezieae (Texas to S. America): <i>Alophia</i> (5), <i>Cypella</i> (30), <i>Ennealophus</i> (5), <i>Gelasine</i> (6), <i>Tigridia</i> (55); <i>Trimezia</i> (12)	split btw. tribes: <35 Myr, Tigridieae crown group: <15 Myr	<i>Centris</i> , <i>Paratetrapedia</i> <i>Tetrapedia</i>	Vogel (1974), Lee (1994)/Goldblatt <i>et al.</i> (2008), (Reis <i>et al.</i> 2000, 2003): Trimezieae and Tigridieae are sister taxa, and the plesiomorphic state for the clade is oil secretion (P. Goldblatt 2009, personal communication)
Krameriaceae: <i>Krameria</i> (18, N. and S. America)	<i>Krameria</i> crown group: 12 (34–5) Myr	<i>Centris</i> ,	Vogel (1974), Simpson <i>et al.</i> (1979); Machado <i>et al.</i> (1997)/this study
Malpighiaceae (1250 total, mostly neotropical, the 250 African and few Asian species lack oil glands)	family crown: 75–64 Myr	<i>Centris</i> , <i>Epicharis</i> <i>Paratetrapedia</i> , <i>Tetrapedia</i>	Vogel (1974, 1988, 1990), Anderson (1979), Raw (1979) and Sazima & Sazima (1989)/Davis <i>et al.</i> (2004): oil glands ancestral in family, at least six losses in African clades
Orchids I: Cranichideae, Cranichidinae: <i>Ponthieva</i> (40), at least <i>P. racemosa</i> , <i>P. tunguraguae</i> and <i>P. maculata</i> offer oil	?	?	Dressler (1993) and G. Gerlach 2009, personal communication. Phylogeny see Salazar <i>et al.</i> (2009)
Orchids II: Cymbidiaceae; Cyrtopodiinae: <i>Grobysa</i> (5, at least <i>G. galeata</i> with oil)	?	<i>Paratetrapedia</i>	Pansarin <i>et al.</i> (2009)
Orchids III: Maxillarieae, Bifrenariinae: <i>Rudolfiella</i> (2, Neotropics)	?	?	Singer <i>et al.</i> (2006), Stpiczynska & Davies (2008) and G. Gerlach 2009, personal communication
Orchids IV: Maxillarieae, Oncidiinae, precise phylogenetic placement unknown, probably several independent origins: <i>Caluera</i> (2), <i>Centroglossa</i> (5), <i>Cyrtochilum</i> (1?), <i>Dunstervillea</i> (1), <i>Platyrrhiza</i> (1), <i>Rauhiella</i> (3), <i>Thysanoglossa</i> (2)	?	<i>Centris</i> , <i>Paratetrapedia</i> , <i>Tetrapedia</i>	Vogel (1974, 1983), Bustos Singer & Cocucci (1999) and Pansarin <i>et al.</i> (2009). Phylogenetic information for these genera from Whitten 2009, personal communication
Orchids V: Maxillarieae, Oncidiinae: <i>Chytroglossa</i> (3), <i>Hintonella</i> (1), <i>Ornithocephalus</i> (46) unknown how many offer oil	?	<i>Paratetrapedia</i> on species of <i>Ornithocephalus</i>	Dressler (1993), van der Cingel (2001) and Silveria (2002)
Orchids VI: Maxillarieae, Oncidiinae: <i>Eloyella</i> (7, unknown how many offer oil)	?	?	Dressler (1993)
Orchids VII: Maxillarieae, Oncidiinae: <i>Oncidium</i> (incl. <i>Sigmatostalix</i> p.p.), <i>Gomesa</i> (1), additional clades likely also offer oil	?	<i>Paratetrapedia</i>	Reis <i>et al.</i> (2000) (for 1 sp.) and Stpiczynska <i>et al.</i> (2007) (for 2 spp.), Aliscioni <i>et al.</i> (2009), Chase <i>et al.</i> (2009)
Orchids VIII: Maxillarieae, Oncidiinae: <i>Trichocentrum</i> , at least <i>T. stipitatum</i> offers oil	?	<i>Centris</i>	van der Cingel (2001) and Silveria (2002)

(Continued.)

Table 1. (Continued.)

independent origins of oil-offering flowers; genera with species numbers and geographical range	age of clade (stem or crown as indicated) ^a	characteristic oil-foraging bees	selected references on: pollination interaction/plant clade age
Orchids IX: Maxillarieae, Oncidiinae: <i>Phymatidium</i> (10, unknown how many offer oil)	?	?	Reis <i>et al.</i> (2006)
Orchids X: Maxillarieae, Oncidiinae: <i>Zygostates</i> (ca 20, unknown how many offer oil)	?	?	Singer <i>et al.</i> (2006)
Plantaginaceae: <i>Angelonia</i> (25), <i>Basistemon</i> (8), <i>Monttea</i> (3), the three genera form a clade that includes the oil-less <i>Melosperma</i> (Oxelman <i>et al.</i> 2005). <i>Monopera</i> (2 spp.) probably also belongs in this clade	split btw. <i>Angelonia</i> and <i>Monttea</i> : 13 (34–7) Myr	<i>Centris</i> , <i>Caenonomada</i> , <i>Paratetrapedia</i> , <i>Tapinotaspis</i>	Vogel & Machado (1991), Machado <i>et al.</i> (2002) (<i>Angelonia</i>), Sersic & Cocucci (1999) (<i>Monttea</i>), Vogel & Cocucci (1995) (<i>Basistemon</i>) and Aguiar & Melo (2009) (<i>Monopera</i>)/this study
Solanaceae: <i>Nierembergia</i> (21). Oil lost in several species	split btw. <i>Nierembergia</i> and <i>Petunia</i> : 12 (38–10) Myr	<i>Centris</i> , <i>Chalepogenus</i> , <i>Tapinotaspis</i>	Neff & Simpson (1981) and Cocucci (1991). Phylogeny see Tate <i>et al.</i> 2009/this study
<i>Holarctic region</i>			
Myrsinaceae: <i>Lysimachia</i> (190, Holarctic, 75 with oil; oil lost several times, e.g. Hawaii endemic subgenus <i>Lysimachiopsis</i>)	crown group: 31 (41–8) Myr	<i>Macropis</i>	Vogel (1976, 1986, 1988). Phylogeny see Hao <i>et al.</i> (2004) and Anderberg <i>et al.</i> (2007)/this study
<i>Africa</i>			
Cucurbitaceae: <i>Momordica</i> (50, Africa and trop. Asia), oil lost in at least two clades; <i>Siraitia</i> (3–4, Asia); <i>Telfairia</i> (3, Asia); <i>Thladiantha</i> (25, Asia) <i>Baijiana</i> (incl. <i>Sinobaijiana</i>) (5, Asia)	stem age: 46 (55–40) Myr, crown group: 22 (34–12) Myr	<i>Ctenoplectra</i>	Vogel (1981a,b) and H. Schaefer 2008, personal observation. Tanzania (2005), Yunnan (2005), Nigeria (2006) and Australia (2007)/Schaefer <i>et al.</i> (2009)
Iridaceae III: Tritoniopsidae: <i>Tritoniopsis</i> (24, S. Africa), only <i>T. parviflora</i> oil-offering	stem age: ca 22 Myr	<i>Redivia</i>	Manning & Goldblatt (2002)/Goldblatt <i>et al.</i> (2008)
Orchids XI: Diseae, Coryciinae I (all S. Africa): <i>Ceratandra</i> (6), <i>Corycium</i> (14), <i>Evotella</i> (1), <i>Pterygodium</i> (19)	?	<i>Rediviva</i>	Pauw (2006) and Whitehead <i>et al.</i> (2008). Phylogeny see Waterman <i>et al.</i> (2009)
Orchids XII: Diseae, Coryciinae II: <i>Disperis</i> (74 Africa, few species in Asia). The Asian spp. and some others without oil	?	<i>Rediviva</i>	Steiner (1989a), Pauw (2006) and Waterman <i>et al.</i> (2009)
Orchids XIII: Diseae, Satyriinae: <i>Satyrium</i> (91, S. Africa, Madagascar, 4 in Asia; only <i>S. rhynchanthum</i> oil-offering)	stem group: 28–31 Myr	<i>Rediviva</i>	Johnson (1997), Whitehead & Steiner (2001)/Verboom <i>et al.</i> (2009)
Scrophulariaceae I: <i>Alonsoa</i> (16 Neotropic, 2 in Africa). Only the African species with oil glands	split btw. <i>Alonsoa</i> and <i>Nemesia</i> : 47.5–42 Myr ¹ or 28 (40–17) Myr ²	<i>Rediviva</i>	Steiner (1989b)/ ¹ Datson <i>et al.</i> (2008) and ² this study
Scrophulariaceae II: <i>Colpias</i> (1, S. Africa, <i>C. mollis</i>)	?	<i>Rediviva</i>	Steiner & Whitehead (2002)
Scrophulariaceae III: <i>Diascia</i> (48, S. Africa)	<i>Nemesia</i> split from <i>Diascia</i> : 32–26 Myr or 15 (24–4) Myr	<i>Rediviva</i>	Vogel (1984), Steiner & Whitehead (1988, 1990, 1991), Pauw (2006)/ ¹ Datson <i>et al.</i> (2008), ² this study
Scrophulariaceae IV: <i>Hemimeris</i> (4, S. Africa)	<i>Hemimeris</i> + <i>Diclis</i> are sister to <i>Alonsoa</i>	<i>Rediviva</i>	Whitehead & Steiner (1985) and Pauw (2005). Phylogeny see Oxelman <i>et al.</i> (2005)
Stilbaceae (all S. Africa): <i>Anastrabe</i> (30), <i>Bowkeria</i> (5), <i>Ixianthes</i> (1)	Stilbaceae stem age: 48 Myr ¹ , split btw. <i>Ixianthes</i> and <i>Nuxia</i> : 17 (18–3) Myr ²	<i>Rediviva</i>	Steiner & Whitehead (1990, 1991) (<i>Bowkeria</i>), Steiner (1993), Steiner & Whitehead (1996) (<i>Ixianthes</i>), Whitehead & Steiner (1992) (<i>Anastrabe</i>)/ ¹ Wikström <i>et al.</i> (2001) and ² this study

^aSuperscript numerals 1 and 2 refer to references 1 and 2 cited in column 4.

Figure 1. Maximum-likelihood phylogeny obtained from 626 *rbcL* sequences representing 440 families of angiosperms (Angiosperm Phylogeny Group 2003), with an over-sampling of oil-offering species. The Iridaceae *Sisyrinchium montanum* is not known to offer oil (Cocucci & Vogel 2001), but this species is the only *Sisyrinchium* for which an *rbcL* sequence was available. Of known oil-offering taxa, the phylogeny lacks the Plantaginaceae *Basistemon* and *Monopera*, the Scrophulariaceae *Colpias*, the Stilbaceae *Anastrabe* and *Bowkeria*, and numerous oil-offering orchids (listed in table 1). The tree therefore does not reflect the true number of independent origins of oil in the angiosperms (detailed version provided as figure 1_large in the electronic supplementary material).

Most of the clades producing floral oil subsequently lost this trait in one or several lineages, with the possible exception of Krameriaceae. We know of the following 36–40 independent losses of oil: (i) In *Calceolaria*, at least 49 of *ca* 260 species lack oil in their flowers (Sérsic 2004). Not all of these have been included in molecular phylogenies, but in a tree that includes 103 species, 22 lack elaiophores, implying six independent losses (A. Cocucci 2009, Universidad Nacional de Córdoba, Argentina, personal communication). These six losses include one in the ancestors of *Stemotria*, a group that is nested in *Calceolaria*. The sister clade of *Calceolaria* is the non-oil-offering genus *Jovellana*, with a few species in South America and two in New Zealand. (ii) In Iridaceae, Trimezieae and Tigridieae are sister taxa, and based on the topology (Goldblatt *et al.* 2008), the most parsimonious explanation for the distribution of oil glands is that they evolved once in the common ancestor of these tribes and that oil secretion was lost at least six times. Even within *Tigridia* there have been multiple losses in groups that switched to bird pollination or buzz pollination by pollen-collecting bees (Goldblatt *et al.* 2008; P. Goldblatt 2009, personal communication). (iii) A phylogeny of the neotropical Iridaceae *Sisyrinchium* implies that

glandular trichomes evolved three times in this genus: once in an early diverging group with few species and twice in somewhat larger clades, followed by a few losses. Inferring their precise number requires a more resolved phylogeny (O. Chauveau 2009, personal communication). (iv) In the Malpighiaceae, for which oil glands are a synapomorphy (Anderson 1979, 1990), oil was lost minimally six times in the ancestors of today's African clades, which together comprise about 250 species (Davis *et al.* 2004; C. Davis 2009, personal communication). Several neotropical Malpighiaceae have also lost floral oil glands (Anderson 1979; Vogel 1990), and oil gland presence can vary even at the population level (Sazima & Sazima 1989). (v) In the Myrsinaceae *Lysimachia*, Vogel (1986, 1988) studied 105 of 189 species, finding evidence for floral oil in 75 of them. Of the 189 species, a molecular phylogeny by Hao *et al.* (2004) includes 57, another by Anderberg *et al.* (2007) includes 86. Based on this sampling and the tree topologies, a minimum of six losses or, alternatively, four gains and two losses can be inferred, depending on whether oil glands are ancestral in *Lysimachia* or not. Examples of the loss of oil in *Lysimachia* include the Hawaiian subgenus *Lysimachiopsis* (Vogel 1986) and species in the South African subgenus

Table 2. Species numbers in clades with and without floral oil. The comparisons for *Calceolaria*, Malpighiaceae, *Lysimachia*, *Nierembergia* and possibly others are not strictly valid since these clades vary for the trait of interest (floral oil). The correct comparisons would be between the subclades that lost floral oil and their sister clades.

clade with floral oil (species number)	clade without floral oil (species number)	data supporting sister relationships
<i>Calceolaria</i> (260), but at least 49 species lack oil <i>Krameria</i> (18)	<i>Jovellana</i> (2–4) Zygophyllaceae (285)	Andersson (2006) Soltis <i>et al.</i> (1998) and Savolainen <i>et al.</i> (2000) Davis & Chase (2004)
Malpighiaceae (1250), but a clade of 250 species lacks oil	Elatinaceae (35)	
<i>Lysimachia</i> (190), but only 75 with oil, and oil then lost several times	sister unclear, perhaps <i>Trientalis</i> (5–6?)	Anderberg <i>et al.</i> (2007)
<i>Angelonia</i> (25), <i>Basistemon</i> (8), <i>Monttea</i> (3), the three genera form a clade that includes the oil-less <i>Melosperma</i> . <i>Monopera</i> (2) probably also belongs in this clade	<i>Ourisia</i> ? (30–40) (but very poor sampling, so relationship not finally resolved)	Oxelman <i>et al.</i> (2005)
<i>Nierembergia</i> (21), but oil lost in several species	<i>Bouchetia</i> (3) + <i>Hunzikeria</i> (3)	Tate <i>et al.</i> (2009)
<i>Telfairia</i> (3)	<i>Ampelosicyos</i> clade (5)	Schaefer <i>et al.</i> (2009)
<i>Colpasia</i> (1)	<i>Hemimeris</i> (8) + <i>Diclis</i> (9) + <i>Alonsoa</i> (14)	Oxelman <i>et al.</i> (2005)
<i>Diascia</i> (48)	<i>Nemesia</i> (65)	Oxelman <i>et al.</i> (2005)
<i>Hemimeris</i> (4)	<i>Diclis</i> (9)	Oxelman <i>et al.</i> (2005)
Stilbaceae: <i>Anastrabe</i> (30), <i>Bowkeria</i> (5), <i>Ixianthes</i> (1)	<i>Nuxia</i> (15) + <i>Stilbe</i> (8) + <i>Retzia</i> (5) + <i>Euthystachys</i> (1) + <i>Campylostachys</i> (2)	Oxelman <i>et al.</i> (2005)

Palladia, which have switched to bird pollination (Sérsic & Cocucci 1996). (vi) In the Plantaginaceae, the oil-offering clade (*Angelonia*, *Basistemon*, *Monopera* and *Monttea*) also includes the oil-less *Melosperma* and a few oil-less species of *Basistemon* (Barringer 1985; Oxelman *et al.* 2005), which implies at least two losses of oil glands. *Monopera* has not yet been sequenced, but morphologically resembles *Angelonia* (Barringer 1983; Aguiar & Melo 2009). (vii) In the Solanaceae *Nierembergia*, oil was lost at least four times (Tate *et al.* 2009; A. Cocucci 2009, personal communication). (viii) In the Cucurbitaceae, oil glands evolved probably only once and were then lost at least six times (H. Schaefer & S. S. Renner 2008, unpublished data).

Table 2 shows species numbers in sister clades with and without floral oil. Unfortunately, a statistical sign test is not yet possible because of insufficient phylogenetic knowledge; a particular problem is that all larger clades (*Calceolaria*, Malpighiaceae, *Lysimachia*, *Nierembergia*, etc.) are variable for the trait of interest (floral oil; see the previous paragraph on the losses of oil in these clades). So far, there is no obvious trend for oil flower clades to be particularly species rich or species poor.

(b) Times of origin of floral oil offering

As shown in table 1, the evolution of oil production in flowers has occurred from the K/T boundary onwards, from the oldest inferred gains in the Malpighiaceae (75–64 Myr) and Cucurbitaceae (49 Myr (confidence interval 57–42 Myr)) to the youngest in Calceolariaceae, Iridaceae, Orchidaceae at 12 to 1 Myr. The age of 41 Myr (52–28 Myr) for the *Lysimachia* stem

lineage (table 1) fits with a possible coevolution between *Palaeomacropis* and *Lysimachia* (§4).

(c) Times of origin of oil foraging in bees

Table 3 lists all known oil-foraging bee genera, with their geographic distribution, number of species and fossil-documented or molecular-clock inferred ages. Overall, some 360–370 species of bees collect oil (table 3), and the behaviour evolved both in the long-tongued and in the short-tongued bees, with the geologically earliest known oil foragers occurring in the Ctenoplectrini and Melittini (figure 2 and table 3). A recent phylogeny of the Melittidae (Michez *et al.* 2009b) suggests the relationships (Macropidini (Melittini [*Rediviva*, *Melitta*])), with *Redivivoides* embedded in *Rediviva*. Assuming that gains and losses of oil-collecting behaviour are equally probable, Michez and coworkers prefer a scenario of independent origins of oil-collecting structures in *Rediviva* and Macropidini over a scenario involving a single origin of oil collecting followed by multiple losses (Steiner & Cruz (2006) prefer the second scenario). They also infer that Macropidini are the sister lineage to *Palaeomacropis eocenicus*, a 53 Myr old bee from France with the typical setae that help *Macropis* take up and transport floral oil (Michez *et al.* 2007). Oil foraging was clearly lost at least once, in *Redivivoides*.

Oil foraging evolved a third time in the paleotropical Ctenoplectrini (figure 2), with two genera that comprise 9 species in tropical Africa and at least 10 in Asia and Australia. All species of *Ctenoplectra* collect floral oil, pollen and nectar from Cucurbitaceae, while their sister clade, *Ctenoplectrina* with three species, is kleptoparasitic and either lost the oil-foraging

Table 3. A list of all known oil-foraging bees with their geographic distribution, number of species and fossil-documented or molecular-clock inferred ages.

independent origins of oil foraging; genera with species numbers and geographical range	oldest fossils or molecular clock-based age (CI)	selected references on bee behaviour, phylogeny and clade ages
<i>New World</i>		
Apidae, Centridini: <i>Centris</i> (230, 30 of these not foraging on oil)	expected to be as old as Malpighiaceae	Vogel (1974), Neff & Simpson (1981) and Sazima & Sazima (1989). For molecular phylogeny: S. Cardinal <i>et al.</i> Cornell University, ongoing
Apidae, Centridini: <i>Epicharis</i> (14–23)	expected to be as old as Malpighiaceae	Vogel (1974), Neff & Simpson (1981) and Sazima & Sazima (1989). For phylogeny: see <i>Centris</i>
Apidae, Tapinotaspini: <i>Arhysoceble</i> (5), <i>Caenonomada</i> (3), <i>Chalepogenus</i> (21), <i>Lanthanomelissa</i> (5), <i>Monoeca</i> (8–9), <i>Paratetrapedia</i> (30), <i>Tapinotaspis</i> (3), <i>Tapinotaspoides</i> (4), <i>Trigonopedia</i> (4)	monophyly?	Vogel (1974), Neff & Simpson (1981) and Sazima & Sazima (1989). For molecular phylogeny: Antonio Aguiar, Museu de Zoologia de São Paulo, <i>Tapinotaspini</i> , ongoing
Apidae, Tetrapediini: <i>Tetrapedia</i> (13)	?	Vogel (1974, 1988), Neff & Simpson (1981) and Alves dos Santos <i>et al.</i> (2002)
<i>Old World or Holarctic</i>		
Apidae, Ctenoplectrini: <i>Ctenoplectra</i> (16)	stem age <i>Ctenoplectra</i> : 56 (67–44) Myr	Vogel (1981a,b) and Schaefer & Renner (2008)
Melittidae, Macropidini: <i>Macropis</i> (16, Holarctic)	<i>Palaeomacropis</i> , 53 Myr	fossil: Michez <i>et al.</i> (2007). For molecular phylogeny see Michez <i>et al.</i> (2009b)
Melittidae, Melittini: <i>Rediviva</i> (24)	?	Vogel (1984), Vogel & Michener (1985) and Steiner & Cruz (2009). For molecular phylogeny see Steiner & Cruz (2006)

behaviour or never had it. Tree topology and molecular dating together suggest that Ctenoplectrini originated in Africa in the Early Eocene and that *Ctenoplectra* dispersed twice from Africa to Asia, sometime in the Late Eocene, 40 to 30 Myr, from where one species reached the Australian continent via Indonesia and New Guinea in the mid-Miocene, ca 13 Myr (Schaefer & Renner 2008).

The fourth, fifth and sixth origins of oil foraging occurred in Centridini (*Centris*, *Epicharis*), Tapinotaspini (*Arhysoceble*, *Caenonomada*, *Chalepogenus* (including *Lanthanomelissa*), *Monoeca*, *Paratetrapedia*, *Tapinotaspis*, *Tapinotaspoides* and *Trigonopedia*), and Tetrapediini (*Tetrapedia*; Michener 2007; our figure 2 and table 3). Tetrapediini include one other genus, *Coelioxoides*, which consists of three species that are kleptoparasitic on *Tetrapedia* (Michener 2007), and perhaps this genus lost oil foraging similar to the situation of *Ctenoplectra* and *Ctenoplectrina* (above). It now appears that Centridini are not monophyletic (S. Cardinal 2009, personal communication), implying a possible seventh origin of oil foraging or, alternatively, losses of the behaviour (reliable interpretation requires a densely sampled phylogeny for *Centris*). Whether Tapinotaspini and Tetrapediini are sister groups or not, thus representing one or two origins of oil foraging, is unclear; a molecular phylogeny that included one representative of most tribes in the subfamily Apinae (Schaefer & Renner 2008) found them to be distantly related, but denser sampling is necessary to test this. Michener (1944: 233 cited in Vogel 1974) assumed that *Centris*, *Epicharis* and the Exomalopsini (now reclassified partly in Tapinotaspini) originated in the Upper Cretaceous, and given that they all interact with Malpighiaceae, in which oil glands are a synapomorphy,

we expect that the stem lineages of these bees go back to at least 64 Myr (table 1).

4. DISCUSSION

(a) *Ages of oil-offering plant clades and oil-foraging bees, and their implications for gradual host niche broadening*

Oil as a pollinator reward evolved at least 28 times independently, with the respective clades currently classified in 11 families and comprising 1500–1800 oil-offering species (table 1). The initial studies on floral oil (Vogel 1969, 1974, 1981a,b, 1988, 1990) and subsequent reviews (Neff & Simpson 1981; Buchmann 1987; Endress 1994; Rasmussen & Olesen 2000; Machado 2004) have all stressed that oil rewards evolved many times independently, as evident from the different types of epithelial and trichomatic oil glands on the sepals, petals, tepals and stamens of the various oil-offering flowers. This is the first review, however, to provide a temporal context and to show that oil was lost more often than it was gained. Estimates for the times of initial divergence are available for two-thirds of the plant and bee clades involved (tables 1 and 3). As predicted by Vogel (1974), the Malpighiaceae are among the oldest clades to have acquired oil glands, and their explosive diversification must have played a key role in the evolution of oil-foraging behaviours in Paleocene and Eocene American bees. From the malpigh fossil record it is evident that the family was widespread in Eocene and Oligocene North America and probably also Europe (Taylor & Crepet 1987; Davis *et al.* 2004 for a summary), but it is not clear whether European malpighs were pollinated by oil-collecting bees.

Figure 2. Maximum parsimony tree showing the relationships of the most important bee groups (modified after [Danforth *et al.* 2006](#), based on five nuclear DNA regions plus morphological data). Eucerini, Tapinotaspidini and Tetrapediini were not sampled in the original study, but are here added based on the results of [Schaefer & Renner \(2008\)](#). Oil foraging lineages in grey.

The Holarctic (Laurasian) *Lysimachia*/*Macropis* system is roughly as old as the malpigh/*Centris* system. This is clear both from the 53 Myr old fossil oil-collecting bee *Palaeomacropis eocenicus* from France, one of the oldest fossils of bees so far discovered (Michex *et al.* 2009a) and the molecular clock-based stem age of *Lysimachia* (41 (28 to 52) Myr).

Based on this temporal coincidence it seems plausible that *Macropis* and *Lysimachia* coevolved from the onset. Today, *Macropis* is strictly dependent on *Lysimachia* from which it obtains not only oil but also pollen, while taking nectar from a range of flowers (Vogel 1986; Michez & Patiny 2005; Michez *et al.* 2008). The latter two works include maps showing

Figure 3. Scheme showing the gradual build-up of oil flower/oil bee interactions in two of the World's four regional oil flower systems. The Eurasian *Macropis/Lysimachia* system and the African/Asian *Ctenoplectra/Cucurbitaceae* system did not involve switches to hosts outside these plant clades. The latter system, however, involved the repeated acquisition of new cucurbit hosts through time, a finer scale host expansion not shown here.

geographic distributions and detailed host–plant associations of *Macropis* and *Lysimachia*.

The ages inferred for the crown groups of *Calceolaria* (8 to 1 Myr), *Krameriaceae* (12 Myr) and various South African orchids (*Satyrium*, 31 to 28 Myr), *Iridaceae* (e.g. *Tritoniopsis*, 22 Myr), *Scrophulariaceae* and *Stilbaceae* (table 1; this also shows confidence intervals around estimates) are relatively young, and individual oil-offering species, of course, are even younger. Older oil-foraging bee lineages therefore must have acquired additional oil hosts over evolutionary time, and figure 3 illustrates how this gradual build-up of oil flower/oil bee interactions could have occurred (as inferred from the molecular clock estimates). In two of the world's four regions with oil flowers, host shifts occurred between plant families (figure 3). In the two others, the Eurasian *Macropis/Lysimachia* system and the African/Asian *Ctenoplectra/Cucurbitaceae* system, host shifts did not leave these plant clades, although the latter system involves several finer scale host expansions (H. Schaefer & S. S. Renner 2008, unpublished data).

Most likely host broadening started with occasional exploratory visits to flowers that resembled the oil hosts in colour or scent. This is plausible because oil bees generally interact with several, rather than a single, flower species (Vogel 1974; Steiner & Whitehead 1988; Machado 2004, and numerous studies cited therein). Among the few one-to-one species interactions may be those between *Rediviva*

and some of its hosts (e.g. Steiner & Cruz 2009). Mimicry of *Malpighiaceae* oil flowers by *Orchidaceae* is well documented (Silvera 2002; Reis *et al.* 2007; Pansarin *et al.* 2008; Aliscioni *et al.* 2009; Carmona-Díaz & García-Franco 2009) and may occasionally lead to new oil-offering orchids. Indeed, convergence on a stereotypical syndrome of floral traits, associated with pollination by oil-collecting bees, has been so strong that genera, such as *Oncidium*, which were defined by floral characters, turn out to be grossly polyphyletic (Chase *et al.* 2009). In some *Oncidium*-type flowers, oil is produced in vaguely defined areas and may serve more in mimicking the spectral reflection of malpigh flowers, than being an actual reward (Chase *et al.* 2009). Deciding whether such initially Muellierian, later probably Batesian, mimicry situations explain most evolutionary acquisitions of new oil hosts, however, will require further data on oil bee behaviour. Some oil bees have chemoreceptors on their tarsi for the detection of oil, and oil-collection behaviour is triggered only when the receptors come in contact with an oily surface (Dötterl & Schaffler 2007).

A scenario of gradual evolutionary host niche broadening (indistinguishable from host switching, when prior hosts became extinct), as proposed above for American oil bees (and visually presented in figure 3), also fits the decidedly asymmetric tropical African oil bee/oil flower system, namely that between *Ctenoplectra* and oil-offering *Cucurbitaceae*.

Ctenoplectra originated in the Early Tertiary, apparently in tropical Africa (Schaefer & Renner 2008) and all species are obligate on Cucurbitaceae from which they obtain nectar, pollen and oil (Vogel 1981a,b, 1990; H. Schaefer 2008, unpublished observations for African and Asian Cucurbitaceae). Its sister clade, Eucerini, does not collect oil. Over the course of evolution, *Ctenoplectra* broadened its host spectrum from the *Momordica* clade to the *Thladiantha*, *Siraitia* and *Telfairia* clades (Schaefer *et al.* (2009) includes a phylogeny showing their distant phylogenetic relationships). We know of no loss of oil foraging within *Ctenoplectra*, although its kleptoparasitic sister clade, *Centoplectrina*, may have lost oil foraging (Schaefer & Renner 2008).

(b) *Why did oil-flowers evolve and persist in very few plant clades?*

Some 28 angiosperm lineages offer oil in their flowers, and from phylogenetic relationships it is possible to infer at least 36–40 losses of floral oil (§3, table 1). This raises several questions. Does the dependence on oil-collecting bees for pollination limit the ability of a plant to expand into new habitats or geographic regions, favouring the loss of oil flowers in the most actively diversifying and expanding clades? Our compilation of sister groups with and without floral oil (table 2) failed to yield an answer to this question because sufficiently detailed phylogenies are not yet available. A second question raised by the few origins, but numerous losses, of oil is how evolutionarily ‘difficult’ it is for a plant to produce oil in floral epithelia or trichomes. What are the biochemical or energetic constraints on floral oils? The chemical composition of floral oils has been analysed in only a few species; it ranges from mainly free fatty acids (Buchmann 1987; Vinson *et al.* 1997) to acetyl-glycerol derivatives with β -acetoxyl-fatty acids (Reis *et al.* 2000) to β -acetate-substituted free fatty acids and mono-, di- or triglycerides (Vogel 1974; Simpson *et al.* 1979; Vinson *et al.* 1997; Silvera 2002; Seibold *et al.* 2004; Dumri *et al.* 2008). A study of the floral lipids from *Calceolaria* (Calceolariaceae) and *Krameria* (Krameriaceae) revealed a C_{16} – C_{20} β -acetoxyl-substituted free fatty acids, and an unusual 3-hydroxy fatty acid (Seigler *et al.* 1978). Buchmann’s (1987) analyses of the caloric content of various floral oils revealed considerable variation, with some oils containing fewer calories than fatty pollen. Whether the numerous instances of loss of floral oils relate to energy constraints is therefore unclear. What is clear, however, is that oil glands were often lost with the occupation of new habitats in which oil bees were rare or lacking (e.g. spread to the Hawaiian archipelago, spread into Africa by neotropical malpighs, spread into South America by African *Momordica*). That the presence and absence of oil glands can vary even within species (Sazima & Sazima 1989) illustrates that pollen- or nectar-foraging visitors can take over pollination services from oil bees at ecological as well as evolutionary time scales.

A profound answer to the question of why oil flower/oil bee systems have remained relatively limited evolutionary experiments (with the exception of

neotropical Malpighiaceae) will require a much better understanding of the costs and benefits of oil-collecting for the bees. Most bees (bee larvae) obtain their fat from pollenkitt, which is collected at the same time as pollen grains. Collecting floral oil instead requires dedicated foraging bouts and behaviours that must come at great costs for females. Perhaps this explains why only 360–370 of the 16 000 species of bees collect floral oil.

We dedicate this paper to Stefan Vogel on the 40th anniversary of his discovery of oil flowers (Vogel 1969).

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