

1 **The origin and evolution of pollen transport in bees (Hymenoptera: Anthophila)**

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4

5 **Abstract**

6 The ability to transport pollen from flowers back to the nest represents a key innovation in the
7 evolution of bees from predatory wasp ancestors. Currently, the origin and evolution of pollen
8 transport remains unsettled. Older hypotheses proposed that crop transport was the original
9 mode of pollen transport, but more recent molecular phylogenies have cast doubt on that view.
10 Instead, more recent hypotheses contend that external transport of dry pollen is ancestral in
11 bees. Here, I propose a new hypothesis to explain the origin and subsequent evolution of pollen
12 transport in bees. I propose that pollen transport arose from adult pollen-feeding behavior and
13 that internal transport of pollen is ancestral in bees. This then led to the evolution of external
14 moist transport, which first required a transition step whereby pollen is temporarily accumulated
15 on the venter on a patch of specialized hairs. Finally, external glazed and dry transport evolved
16 from external moist pollen transport, and the evolution of dry transport led to changes in the
17 location of scopae from the original location on the hind tibia and basitarsus. I illustrate many of
18 these hypothetical evolutionary steps using modern-day bee behavior as an example, with a
19 particular focus on the bee *Perdita tortifoliae*. Examination of the evolution of pollen transport of
20 pollen wasps (subfamily Masarinae) reveals that they have undergone a parallel evolutionary
21 change. Overall, I lay out a broad hypothetical framework to explain the origin and subsequent
22 evolution of pollen transport in bees. This marks a return to the earlier hypothesis that crop
23 transport is ancestral, and it also represents the first in-depth hypothesis to explain how external
24 transport of moistened pollen could have evolved. The evolutionary history of bees has many
25 implications for the biology of bees in the present day, and I lay out a number of predictions that
26 could help confirm or refute my hypotheses.

27 **Keywords:** Apoidea, pollen gathering, pollen feeding, *Perdita*, Masarinae

28 Introduction

29 The evolution of bees from predatory wasp ancestors is one of the major evolutionary
30 developments within Hymenoptera. The shift from a predatory to vegetarian lifestyle may
31 explain the relatively rapid diversification of bees compared to their closest relatives (Branstetter
32 et al. 2017, Murray et al. 2018). In addition, the origin of bees approximately 120 mya coincides
33 with the diversification of early flowering plants (Cardinal and Danforth 2013). However, the
34 mechanisms of how bees originally evolved from predatory wasps remains shrouded in mystery.
35 In particular, the shift from hunting prey to gathering and transporting pollen would require major
36 changes in both morphology and behavior.

37 The lack of information on the biology of close relatives of bees, combined with the lack of
38 fossils of early bee lineages, make reconstructing the genesis and evolution of bees difficult
39 (Engel 2001, Michez et al. 2012). Recent molecular phylogenies of Hymenoptera suggest
40 pemphredonine wasps are the sister group to bees (Debevec et al. 2012, Branstetter et al.
41 2017, Peters et al. 2017, Zheng et al. 2018). Sann et al. (2018, 2021) pointed to the
42 Ammoplanidae (also known as the pemphredonine subtribe Ammoplanina) as the closest
43 relatives of bees. The biology of relatively few species of Ammoplanidae are known. These
44 wasps are often found on flowers and the few species for which the biology is known provision
45 nests with Thysanoptera (Maneval 1939, Bohart and Grissell 1972). Other related groups
46 provision with Hemiptera, Collembola, or Thysanoptera (Bohart and Menke 1976). Since the
47 prey preferences of such a small number of species are not necessarily representative of all the
48 species in the group, this tells us little information other than the ancestor of bees potentially
49 visited flowers to find prey and needed to use many prey items (and thus provisioning trips) to
50 complete their provisions (Malyshev 1969).

51 One of the most important unanswered questions regarding the genesis of bees is how they
52 evolved to transport pollen from flowers back to the nest. In the present day, there exist multiple
53 modes of pollen transport. Bees can transport pollen either internally (in the crop) or externally
54 on specialized structures composed of specialized hair brushes (scopae) or flattened plates
55 (corbiculae) (Thorp 1979). External pollen transport can be further broken down into three
56 modes: pollen can be transported completely dry, completely moist, or glazed, where moist
57 pollen is packed on top of dry pollen (Portman and Tepedino 2017). The evolutionary sequence
58 between the different modes of pollen transport is currently unsettled and it is not clear why
59 multiple modes of pollen transport exist.

60 The traditional hypothesis has been that the original bees transported pollen internally in the
61 crop (Müller 1883, Malyshev 1936, 1969, Michener 1965, 1979, Jander 1976, Lanham 1980).
62 However, that hypothesis was based in part on the idea that the family Colletidae was basal due
63 to its short, bilobed glossa, a character shared with many Sphecidae wasps (Michener 2007).
64 Because many Colletidae transport pollen in the crop (e.g. Euryglossinae and Hylaeinae), this
65 offered a simple and straightforward solution to the problem of how ancestral, wasp-like bees
66 transported pollen (Michener 1979). However, advances in bee phylogenetics have supported
67 Melittidae, rather than Colletidae, as the basal bee family (Danforth et al. 2006, 2012). The
68 family Melittidae contains no known species that transport pollen internally, causing the crop-
69 transport hypothesis to fall out of favor (Michener 2000, Danforth et al. 2019).

70 More recently, the favored hypothesis is that the original bee transported pollen dry on external
71 scopal hairs (Michener 1944, 2000, 2007, Roberts and Vallespir 1978, Radchenko and Pesenko
72 1996, Westerkamp 1996, Engel 2001). The more detailed explanations of this hypothesis
73 propose that the protobee, “the hypothetical most recent common ancestor of all bees”
74 (Michener 2000), carried pollen on unspecialized hairs over most of the body surface, and then
75 over time the generalized body hairs specialized and coalesced into discrete structures
76 (Radchenko and Pesenko 1996, Michener 2007). For example, in some bees they formed
77 scopa on the abdominal venter (the family Megachilidae) while others formed scopa on the hind
78 legs (the families Andrenidae and Halictidae). However, one of the main problems with this
79 hypothesis is that the wasp ancestors of the original bee likely did not have copious body hairs,
80 as the closest wasp relatives to bees, the pemphredonine wasps, are small and largely hairless.
81 More recently, Sann et al. (2018), pointing to the evolutionary relationship with pemphredonine
82 wasps, proposed that the ancestor of bees could have transitioned to pollen provisioning by
83 carrying pollen-covered thrips. However, they did not propose any actual mechanism to explain
84 how that could have led to scopae and the deliberate transport of pollen.

85 Recently, Portman and Tepedino (2017) questioned the hypothesis that external dry transport is
86 ancestral. This was based on an examination of the patterns of evolution of pollen transport in
87 the genera *Perdita* (Andrenidae) and *Hesperapis* (Melittidae); in both genera, it was found that
88 moist pollen transport was the most likely ancestral state and glazed or dry transport were the
89 derived states. This raised the intriguing possibility that external moist transport represents the
90 ancestral state of bees as a whole (Portman and Tepedino 2017). However, we did not propose
91 a potential mechanism for how this could occur, and to date, no studies have proposed an
92 explanation for how moist transport could have evolved, regardless of whether it represents the

93 original pollen transport mode or evolved from another existing pollen transport mode such as
94 dry transport. The only hypothesis I am aware of that even touches on it is by Michener et al.
95 (1978), which suggests the corbiculae in Apidae arose from ancestral “brushy” scopae on the
96 hind legs, potentially as a way to transport sticky nest materials.

97 Finally, the evolution of the protobee can be informed by the parallel evolution of wasps in the
98 vespid subfamily Masarinae, which have also evolved to provision their larvae with pollen. Bees
99 and pollen wasps both arose around a similar time in the mid-Cretaceous (Branstetter et al.
100 2017, Peters et al. 2017). All known masarid wasps transport pollen in the crop, making it
101 unambiguously the ancestral trait. Further, exploring the differences in their evolution can help
102 explain why bees are so much more diverse than masarid wasps, with approximately 20,000
103 species in bees (Michener 2007) vs. approximately 300 species in Masarinae (Carpenter 2001).

104 The purpose of this paper is to address two specific questions: (1) which mode of pollen
105 transport is ancestral in bees? And, (2) how did the ancestral state of pollen transport diversify
106 into the different modes (internal, moist, dry, glazed) seen in the present day? To address these
107 questions, I follow two primary lines of evidence. First, I use present day bee behaviors
108 (specifically pollen transport, pollen gathering, and pollen feeding) to construct hypotheses
109 regarding how pollen transport originated and transitioned from one mode to another. Second, I
110 examine the biology of masarid pollen wasps, which have undergone a parallel transition to
111 pollen provisioning from predatory ancestors. This approach follows the strategies used by
112 Malyshev (1969) and Jander (1976), but my investigation benefits from recent advances in bee
113 phylogenetics and the greatly increased knowledge of apoid and masarid biology.

114 Unexpectedly, my conclusions match those of Malyshev (1969) and Jander (1976) in supporting
115 crop transport as ancestral in bees. I further propose that external transport of moistened pollen
116 evolved from crop transport, and I propose a sequence of steps that could result in that
117 transition. The evolution of external moist transport from crop transport is supported by three
118 primary lines of evidence. First, the behavioral steps involved in moistening pollen for transport
119 involve extraneous steps that appear to represent evolutionary vestiges. Second, the similarity
120 of the behavioral steps involved in eating pollen and moistening pollen suggest a shared
121 evolutionary origin. Third, I examine parallel patterns of evolution that have occurred in masarid
122 wasps that may represent transitional evolutionary steps that occurred in bees. Finally, I
123 propose that external dry transport evolved from moist pollen transport and that this led to the
124 expansion and migration of scopal hairs in many bee lineages.

125 **Methods**

126 Observations of bees took place primarily in Utah and Nevada. *Perdita tortifoliae* Cockerell was
127 observed in the vicinity of St. George Utah, in 2016 and 2017. *Macrotera latior* (Cockerell) and
128 *Hesperapis "timberlake"* (manuscript name from Stage (1966)) were observed in April 2017, in
129 Lake Mead National Recreation Area. Identifications were made with reference the following
130 taxonomic resources: *Perdita tortifoliae*: Timberlake (1968) and comparison to specimens in the
131 Bee Biology Systematics Laboratory (BBSL) collection; *Macrotera latior*: Danforth (1996) and
132 comparison to specimens in the BBSL collection; *Hesperapis "timberlake"* MS name: Stage
133 (1966) and comparison to specimens in the BBSL collection. Representative specimens were
134 collected and are deposited in the BBSL collection. Collections of bees in Lake Mead National
135 Recreation Area were made under permit #LAKE-2017-SCI-0004.

136 A Quanta FEG 650 Scanning Electron Microscope was used to image the specimen hairs and
137 videos were taken with a Sony A65 DSLR camera and edited using Sony Movie Studio 13
138 software.

139 **Results and Discussion**

140 **The roadmap**

141 The basic steps in the origin and evolution of pollen transport follow the general sequence of
142 crop transport -> external moist transport -> external dry transport.

- 143 1. Crop transport represents the original form of pollen transport and evolved from pollen
144 feeding behavior. Bees consumed pollen by nibbling with the mouthparts and by drawing
145 a pollen-covered foreleg through the mouthparts.
- 146 2. The next stage of pollen transport evolution was the accumulation of pollen on the
147 venter. The accumulated pollen was then picked up by the foreleg and brought forward
148 to the mouthparts and consumed by drawing the foreleg through the mouthparts.
- 149 3. Next, external moist pollen transport evolved from internal transport, likely due to leftover
150 pollen becoming stuck to the hind leg rather than completely groomed off.

151 4. External dry and glazed transport evolved from external moist transport in parallel with a
152 development of the scopal hairs, following the hypothesis of Portman and Tepedino
153 (2017).

154 5. Finally, in various lineages that transport dry pollen, the scopal hairs expanded and
155 migrated from the hind tibia and basitarsus towards the midline of the body. In other
156 lineages, crop transport secondarily evolved.

157 What follows is a rather meandering discussion of the evidence supporting this roadmap. This is
158 then compared to the hypothesized parallel evolution of pollen transport in pollen wasps.

159 **Hypothesis: Crop transport is ancestral and it evolved from ancestral adult**
160 **pollen-feeding behavior**

161 In the present day, pollen feeding is an integral part of bee biology; pollen is eaten by adult bees
162 (both male and female) and is necessary for the production of eggs (Robertson 1929, Rozen
163 1989, 1958, Stockhammer 1966, Shinn 1967, Jander 1976, Batra 1985, Hunt et al. 1991,
164 Richards 1994, Michener 2007, Schäffler and Dötterl 2011, Cane 2016, Cane et al. 2016,
165 Houston 2019). While gathering pollen, females will often take a bite to eat without interrupting
166 pollen gathering activities (Jander 1976, ZP pers. obs.).

167 The ubiquity and importance of pollen-feeding in bees suggests a basal origin, and it is simple
168 to hypothesize how ancestral pollen-feeding behavior could evolve into transport of pollen in the
169 crop. In this case, it would require adults of the protobee to first consume pollen and nectar (or
170 other plant exudates) for its own energetic and nutritional needs. Despite the limited fossil
171 record, there is direct evidence that aculeate wasps fed on angiosperm pollen for their own
172 nutritional needs as early as the cretaceous (Grimaldi et al. 2019). The next step in the evolution
173 of pollen transport requires the protobee to regurgitate the consumed pollen and nectar back at
174 the nest. The specific behaviors and mechanisms by which regurgitation evolved are unknown.
175 However, regurgitation of food to provision the young has evolved multiple times in multiple
176 different Hymenopteran lineages including ants, pollen wasps and other vespids (Liebig et al.
177 1997).

178 This hypothesis — that crop transport evolved from pollen feeding — has been previously
179 proposed by Malyshev (1969) and Jander (1976). The strongest argument against it is that
180 there are no known examples of basal bees that transport pollen in the crop. However, there are

181 two points that support this hypothesis: first, essentially all bees that have had their biology
182 explored in depth feed on pollen and regurgitate nectar onto their larval provisions. These two
183 behaviors may represent evolutionary vestiges of ancestral crop transport. Second, as I will
184 explain subsequently, crop transport provides transition steps that are necessary for the next
185 stage of pollen transport: the evolution of external moist transport.

186 **A discussion of the mechanisms by which bees feed on pollen**

187 In order to understand the evolution of pollen transport, it is first necessary to have a thorough
188 understanding of the specific steps bees use to feed on pollen. All known bees consume pollen
189 by drawing the foreleg through the mouthparts (Jander 1976, Michener 2007). Use of the
190 foreleg for consuming pollen represents a modification of typical Hymenopteran grooming
191 behavior. In most other Hymenoptera, the foreleg is cleaned by drawing it through the
192 mouthparts (Farish 1972, Jander 1976). However, in bees, this movement has been co-opted
193 for pollen feeding — indeed, the majority of bee groups have a comb on either the galea or
194 stipes that is specifically used for scraping pollen from the foreleg (Jander 1976). Supporting
195 this hypothesis that ancestral foreleg grooming has been co-opted for pollen feeding is the fact
196 that bees are potentially unique among Hymenoptera in grooming the foreleg by pulling it
197 through the bent midleg (Farish 1972, Jander 1976; see Fig. 3 of Jander 1976 for illustration). In
198 other words, the ancestral method of foreleg-cleaning (drawing it through the mouthparts) has
199 been replaced by a derived method of foreleg-cleaning (drawing it through the crook of the
200 midleg).

201 The use of the foreleg for consuming pollen presents a puzzle since presumably the simplest
202 way to consume pollen would be to nibble it directly with the mouthparts. Indeed, bees are
203 capable of nibbling pollen directly with the mandibles and have been observed to do so when
204 consuming pollen directly from pollen masses in the nest (e.g. Batra 1964), but they apparently
205 do not perform this behavior on flowers (Jander 1976). This is likely because using the forelegs
206 for pollen consumption offers two main advantages: first, it allows consumption of pollen from
207 any place the foreleg can groom, namely the head and thorax (Jander 1976). This allows bees
208 to exploit pollen that has been deposited on the head or thorax by a flower. Second, nibbling
209 pollen presents mechanical difficulty in that pollen is difficult to swallow. In order to be easily
210 swallowed in a large quantity, pollen must be mixed with regurgitated nectar (or potentially some
211 other fluid), a process I have frequently observed performed by bees eating pollen, and pollen
212 feeding behavior is often performed in tandem with nectar concentrating behavior (see Portman

213 et al. In Press). The hypothesis that feeding on pollen requires a liquid such as nectar is
214 supported by the fact that bees dissected after eating pollen have a mix of pollen and nectar in
215 the crop (Danforth 1989, 1990, Cane et al. 2016). Other pollen-feeding Hymenoptera face this
216 same problem but solve it by a different route: Mutillidae and Scoliidae regurgitate liquid directly
217 onto anthers before consuming the pollen (Jervis 1998).

218 **A window back in time: Modern day pollen-feeding behavior is essentially the**
219 **same as ancestral pollen transport behavior.**

220 Eating pollen for adult nutrition and consuming pollen in order to transport it back to the nest are
221 functionally equivalent behaviors. The only real difference is whether or not the bee regurgitates
222 the pollen back at the brood cell (provisioning) or digests it (feeding). As a result, a careful study
223 of the mechanisms by which bees feed on pollen can provide a template for how the protobee
224 transported pollen.

225 Here, I use the bee *Perdita tortifoliae* as the archetypal bee to demonstrate pollen feeding and
226 pollen gathering behavior. I use this bee primarily because I have been able to make a close
227 and careful study of its pollen-feeding and pollen-gathering habits. *Perdita tortifoliae* is a minute
228 bee (about 4 mm body length) that specializes on the pollen of *Lepidium* (Brassicaceae), which
229 it transports moistened on the hind legs. It occurs in the arid western United States and is locally
230 common in the vicinity of St. George Utah, where observations took place.

231 **Pollen-feeding behavior in *Perdita tortifoliae***

232 Similar to other bees, the females of *P. tortifoliae* will occasionally take bites of pollen while
233 gathering pollen and packing it into their scopae. However, towards the end of their daily activity
234 on flowers in the afternoon, *P. tortifoliae* females engage in dedicated feeding trips where they
235 exclusively consume pollen without packing any into the scopa. Indeed, any excess pollen is
236 totally discarded. This feeding trip is presumably the same as feeding trips in other panurgine
237 bees, who return to the nest with empty scopa but have pollen and nectar in the crop (Danforth
238 1989, Neff and Danforth 1991, Visscher and Danforth 1993). These previously observed feeding
239 trips have only been observed through dissecting bees returning to their nests and, to the best
240 of my knowledge, this behavior of feeding on pollen in panurgines has not previously been
241 reported. One of the most important features of the pollen-feeding behavior of *Perdita tortifoliae*
242 is that the bees first accumulate pollen on a specialized patch of hairs on the venter (as in Fig.

243 4A–B), a strategy that is well-documented in the pollen-gathering behavior of various panurgine
244 bees (Portman et al. 2019).

245 The behavior of pollen feeding can be divided up into five main steps (see also Figure 1 and
246 Supplemental Video 1: <https://youtu.be/6M4BpnQ8zfc>):

247 **Step 1. Accumulating pollen:** The forelegs (and occasionally the midlegs) are used to scrape
248 pollen directly from anthers and deposit it on the venter of the thorax.

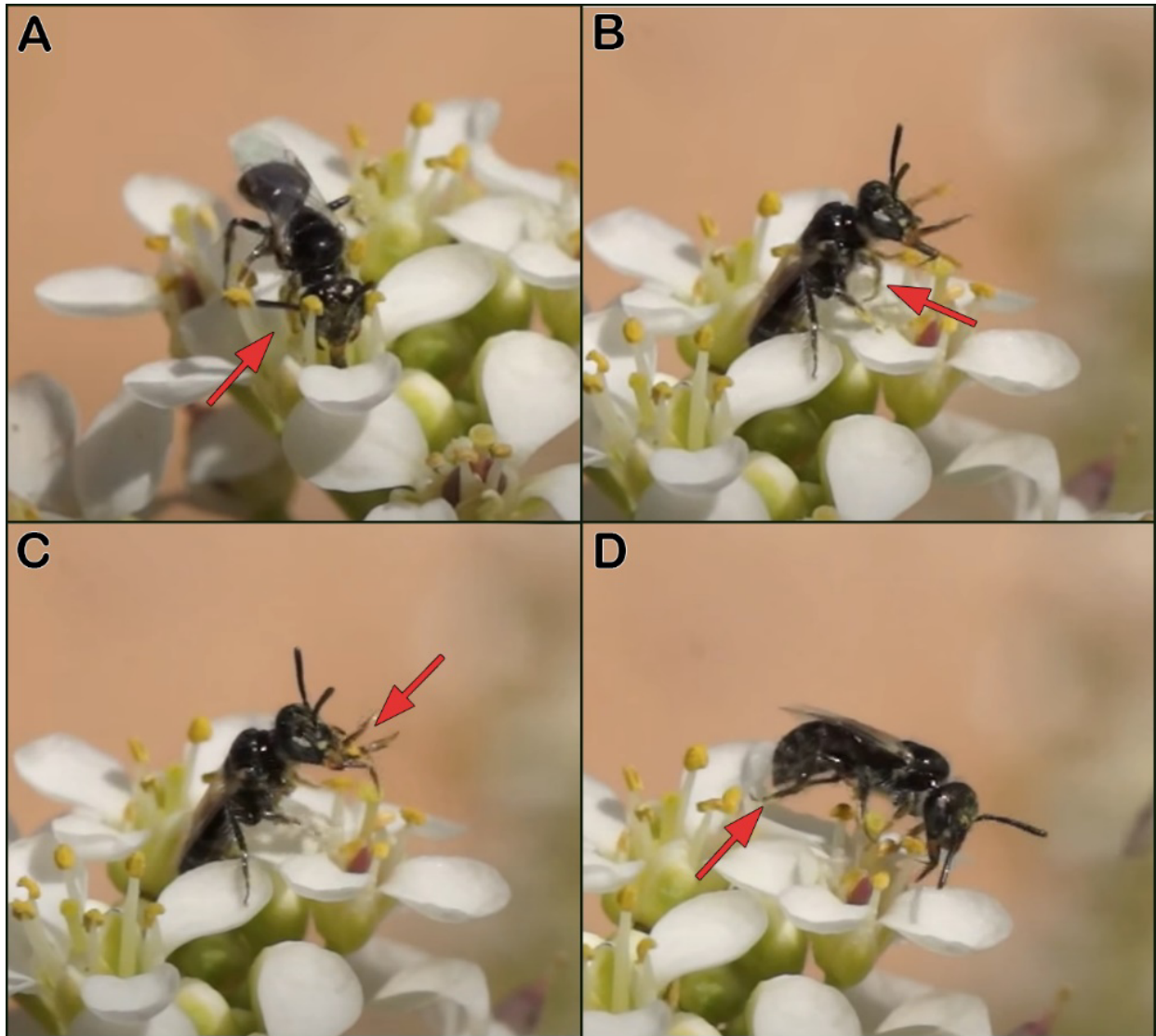
249 **Step 2. Unloading pollen:** After a sufficient quantity of pollen has accumulated on the venter
250 of the thorax, the bee rears back on its hind legs, often forming a tripod with the apex of the
251 abdomen. Pollen is removed from the venter by the forelegs using from one to ten downward
252 scraping motions.

253 **Step 3. Bringing pollen forward:** The legs with pollen are brought to the mouthparts and the
254 tongue is extended, and the bee regurgitates nectar onto the base of the mouthparts.

255 **Step 4: Eating the pollen:** One at a time, each foreleg is drawn through the mouthparts, either
256 in between the split galeae or between the closed galeae and a mandible.

257 Steps 2–4 are then repeated until the pollen has been removed from the venter of the bee.

258 **Step 5: Discarding excess pollen:** During the whole process, excess pollen is continuously
259 groomed off of the front legs by scraping them through the crook formed by the inner side of the
260 mid-femur and mid-tibia, and the midlegs are in turn scraped through the crook of the hindlegs.
261 The pollen is then groomed and discarded by the hindlegs rubbing against each other.



262

263 Figure 1. Pollen-feeding behavior in *Perdita tortifoliae* on *Lepidium* flowers. A) Using the foreleg
264 to accumulate pollen on the venter B) Rearing back and using the forelegs to remove the pollen
265 from the venter. C) Splitting apart the mouthpart and drawing the pollen-covered foreleg through
266 the mouthparts D) Using the hind legs to remove excess pollen. It is much clearer in the video,
267 available as Supplemental video 1: <https://youtu.be/6M4BpnQ8zfc>

268

269 **Hypothesis: this represents the ancestral form of pollen transport.** I propose that this mode
270 of feeding on pollen represents the ancestral form of gathering pollen. It is a key point that the
271 pollen is accumulated on the venter prior to being consumed. It seems probably that the
272 accumulation of pollen on the venter step evolved after pollen transport in the crop, though it

273 could have evolved before crop transport as a way to more efficiently feed on pollen. However,
274 regardless of when it evolved, it is a necessary preadaptation to evolve external pollen
275 transport. This will be made clear by a comparison of pollen-feeding and external pollen
276 transport in *Perdita tortifoliae* in the next section.

277 **The evolution of external moist pollen transport from pollen feeding** 278 **behavior**

279 One of the key points in my argument is that only a couple minor changes are required to turn
280 pollen feeding into external pollen transport. I will demonstrate this here by describing pollen-
281 gathering and packing behavior of *Perdita tortifoliae* and comparing it to pollen-feeding behavior
282 in *P. tortifoliae* that was described in the previous section. I then show how pollen-feeding can
283 evolve into external moist pollen transport with just some minor changes.

284 **Pollen gathering and packing behavior in *Perdita tortifoliae***

285 Like other panurgines, and similar to how it feeds on pollen, *P. tortifoliae* gathers pollen using a
286 two-step process, where it temporarily accumulates pollen on a specialized patch of apically
287 hooked hairs on the venter of the thorax before transferring it to the hind legs (as in Fig. 4A–B;
288 reviewed in Portman et al. 2019). It also moistens the pollen before packing it onto sparse
289 scopae for transport (Portman and Tepedino 2017). Here, I further break it down into finer steps
290 in order to better illustrate the component behaviors (see also Figure 2 and Supplemental video
291 2: <https://youtu.be/v1G96DLynCQ>).

292 **Step 1. Accumulating pollen:** The forelegs (and occasionally the midlegs) are used to scrape
293 pollen directly from anthers and deposit it on the venter of the thorax.

294 **Step 2. Unloading pollen:** After a sufficient quantity of pollen has accumulated on the venter of
295 the thorax, the bee rears back on its hind legs, often forming a tripod with the apex of the
296 abdomen. Pollen is removed from the venter by the forelegs using from one to ten downward
297 scraping motions.

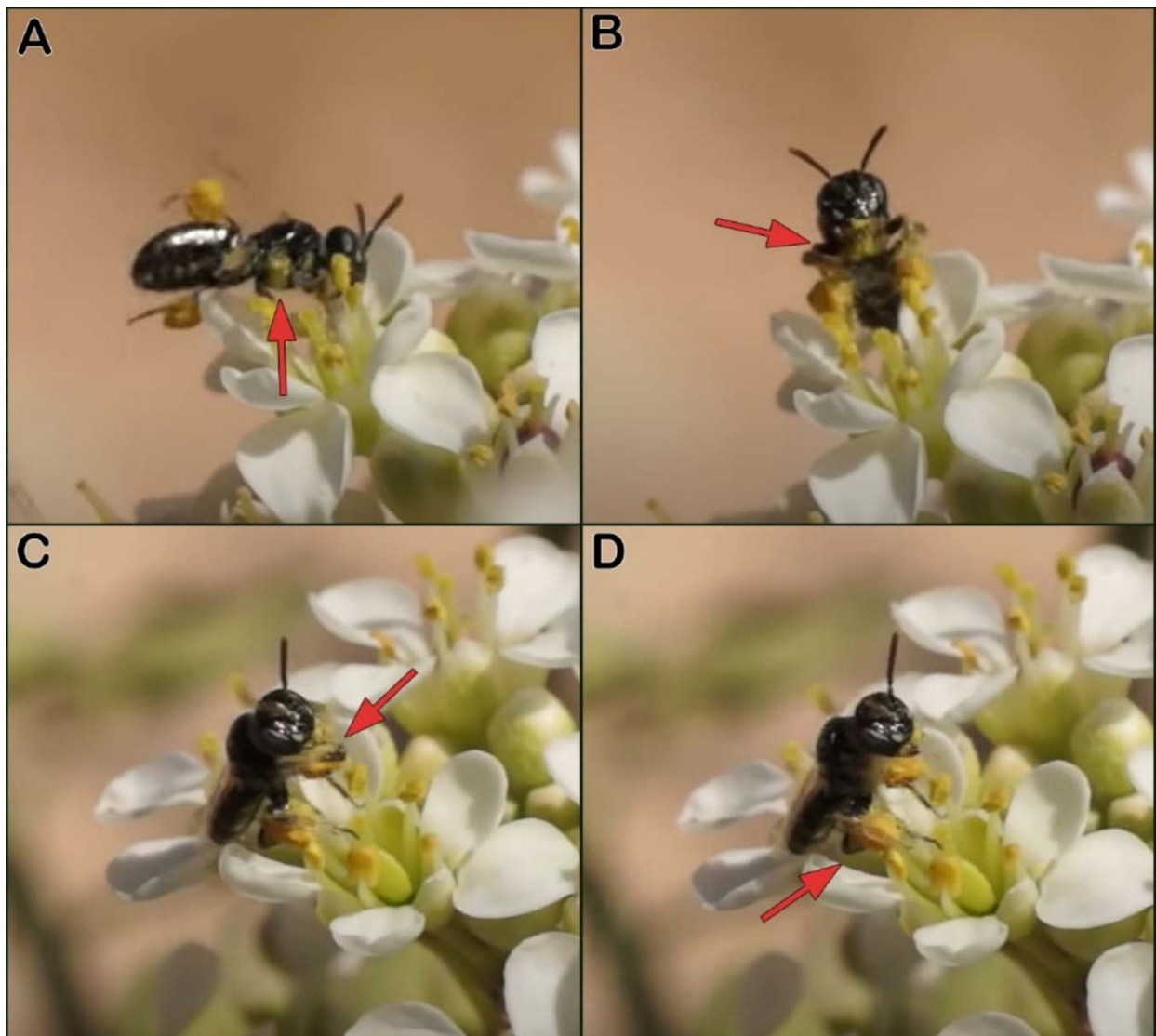
298 **Step 3. Bringing pollen forward:** The legs with pollen are brought to the mouthparts, the
299 tongue is extended, and the bee regurgitates nectar onto the base of the mouthparts.

300 **Step 4: Moistening the pollen:** Both forelegs are brought up together and scraped across the
301 top of the extended mouthparts, moving from the base to the apex of the mouthparts and
302 picking up regurgitated nectar in the process.

303 **Step 5: Transferring and packing the pollen back on the hind legs:** Immediately following
304 pollen moistening, the foreleg is drawn through the midleg, in a crook formed by the inner side
305 of the mid-femur and mid-tibia, causing the pollen to be transferred to the hind part of the
306 midleg, and the midleg then pats back against the hind tibia, depositing the pollen.

307 Steps 3–5 are then repeated until the pollen has been removed from the venter of the bee.

308



309

310 Figure 2. Pollen gathering behavior in *Perdita tortifoliae*. A) Accumulating pollen on the venter.
311 Pollen is visible because the female was just knocked by a male. B) Rearing back and removing
312 the pollen from the venter with the forelegs. C) Drawing the pollen-covered forelegs along the
313 extended mouthparts to moisten them with pollen. D) Using the midlegs to transfer the
314 moistened pollen to the hind legs and tamp it down. It is much clearer in the video, available as
315 Supplemental video 2: <https://youtu.be/v1G96DLynCQ>

316 **Changing to external moist transport from pollen feeding**

317 Comparing pollen feeding to pollen packing behavior in *Perdita tortifoliae* (Table 1), two key
318 points are apparent. First, they represent variations on the same basic behavior, and the first
319 three steps are shared between them. Second, only two changes are needed to go from pollen-
320 feeding to pollen-packing: in pollen-packing, the pollen is brought along the mouthparts to be
321 moistened (rather than consumed) and the pollen is packed onto the hind legs (rather than
322 groomed off). As we have seen, when the bee feeds on pollen, a portion of pollen is already
323 passed along the tongue without being consumed, so packing rather than discarding pollen is
324 the primary step that needs to change.

325

326 Table 1. Comparing the steps of internal transport (pollen feeding) behavior vs external moist
327 transport behavior

	Internal transport	External moist transport
Step 1	Accumulating pollen	Accumulating pollen
Step 2	Unloading pollen	Unloading pollen
Step 3	Bringing pollen forward	Bringing pollen forward
Step 4	Eating the pollen	Moistening the pollen
Step 5	Discarding excess pollen	Accumulating pollen on the hind legs

328

329

330

331 **Evolutionary implications**

332 Viewing this from an evolutionary standpoint, this provides the map for how external pollen
333 transport evolved; as a modification of crop transport. In essence, external pollen transport
334 represents internal pollen transport with just a couple of modified steps. Both behaviors center
335 around the temporary accumulation of pollen on the venter; the main difference being that in
336 pollen-feeding, the pollen is consumed by the mouthparts and in pollen-gathering the pollen is
337 moistened by the mouthparts and passed back.

338 The steps between pollen-feeding and external pollen transport require few to no transition
339 steps. One could imagine a gradual change where internal pollen transport in the crop slowly
340 changes over to external moist transport. In this case, some of the excess pollen from eating
341 would be passed back and glommed onto the legs rather than groomed off completely. Because
342 this pollen had been in contact with the nectar at the mouthparts, it would be moistened and
343 sticky. The bee would instead wait until it was back at the nest to completely groom it off. This
344 amount of pollen on the legs would grow over time, making up a greater and greater proportion
345 of the pollen load, until eventually, external moist transport became the predominant or sole
346 method of pollen transport.

347 Viewing external moist transport as a behavior that has been tacked onto pollen-feeding
348 behavior explains the incongruous step in pollen gathering behavior, where the bee brings the
349 pollen forward to the mouthparts to be moistened. During that process, the bee picks up the
350 pollen from the venter of the thorax, brings the pollen forward to be moistened at the
351 mouthparts, only to immediately pass the pollen backwards towards the hind legs. This stands
352 in contrast to what seems like the more logical method of simply passing the nectar backwards
353 to moisten pollen (as if often seen in honey and bumble bees). However, bringing pollen forward
354 makes sense because that represents the origin of the behavior from when the bee simply ate
355 the pollen that was brought forward rather than moistening it. In this view, passing pollen
356 forward to the mouthparts to be moistened represents a vestige of the ancestral pollen feeding
357 behavior and this step is retained due to its evolutionary history rather than any particular utility.

358 If moist transport evolved from internal transport, it explains how external pollen transport could
359 have evolved without any specialized pollen-carrying structures, as *Perdita* (and many other
360 bees that transport moistened pollen) carry pollen on short, sparse, and simple scopal hairs.
361 Indeed, the protobee may have been similar in many ways to *Perdita tortifoliae*: small, relatively

362 hairless, and lacking specialized pollen transporting hairs. The one exception to the lack of
363 specialized hairs is the specialized patch on the venter. Though I do not go into it here, the
364 temporary accumulation of pollen on a specialized patch of hairs (e.g. Fig. 4) provides a
365 potential mechanism for bees to specialize on the morphological properties of pollen despite the
366 lack of specialization in the scopal hairs; this is important given that pollen specialization is
367 increasingly viewed as the ancestral state in bees (Michez et al. 2008, Sedivy et al. 2008).

368 There are still many unknowns about the exact behavior and evolutionary history of the first
369 bees. It is worth noting the possibility that crop transport is not ancestral, and instead external
370 pollen transport evolved directly from adult pollen-feeding behavior (using the same mechanism
371 just outlined). However, I consider that unlikely, particularly given the parallel evolution of pollen
372 wasps, discussed in a later section. In addition, it seems unlikely that feeding on pollen for adult
373 nutrition would generate enough excess pollen to attach in appreciable quantities on the hind
374 legs, especially in an individual that continues to have a predatory lifestyle. It seems likely that
375 the earliest forms of crop-transporting bees are extinct; given that all known bees — including
376 males and parasitics — share a broadened hind basitarsus (Radchenko and Pasenko 1996,
377 Michener 2007). This suggests that the most recent common ancestor of all extant bees
378 transported moist pollen on the hind legs.

379 **Some additional supporting evidence from other bees**

380 While the behavior of *Perdita tortifoliae* was used to illustrate the proposed evolutionary
381 sequence of steps in the evolution of pollen transport, they are by no means a special case.
382 They are merely the ones I had the opportunity to observe the most in-depth, and there are
383 additional bees that have these same behaviors. For example, the same pollen-feeding and
384 pollen-gathering behaviors were observed in the species *Macrotera latior* (*Macrotera* is the
385 sister genus to *Perdita*), though their faster speed and tendency to transfer the pollen without
386 standing in a tripod position made the behaviors more difficult to observe and record (*M. latior*
387 pollen feeding: Supplemental video 3: https://youtu.be/tdUz_iTr8qY and *M. latior* pollen
388 gathering: Supplemental video 4: <https://youtu.be/l6C6KtmqSD8>). In addition, the practice of
389 temporarily accumulating pollen on the venter is widespread in other panurgine bees, reported
390 in at least 14 other panurgine species, and it has also been recorded in disparate other groups,
391 including *Trigona* and *Macropis* (reviewed in Portman et al. 2019). Although pollen feeding
392 behavior has not been documented for those species, I see no reason why they would differ
393 from *Perdita tortifoliae* and *Macrotera latior*.

394 The same pollen feeding and gathering behaviors also occur in the melittid bee *Hesperapis*
395 “*timberlakei*” Stage (1966) manuscript name (hereafter *H. “timberlakei*”). This bee has a
396 preference for *Psorothamnus* pollen but also gathers pollen from *Larrea* (Michez et al. 2008, ZP
397 pers. obs.). It transports moistened pollen on hind leg scopae (Portman and Tepedino 2017).
398 Multiple females of *H. “timberlakei*” were observed gathering pollen from *Psorothamnus*
399 *fremontii*, and a short clip of one was recorded (Fig. 3, Supplemental video 5:
400 <https://youtu.be/Tpbd2UrmLIs>). These observations confirm two key aspects of the pollen
401 gathering behavior of *H. “timberlakei*”. First, gathered pollen is initially accumulated on the
402 venter of the thorax by the fore- and midlegs. Second, pollen is passed up to the mouthparts to
403 be moistened before being passed back to the scopae (Fig. 3B). Because the transfer of pollen
404 from the venter took place while the bee was in flight, it was very difficult to observe, though the
405 movements can be discerned when the video is slowed down (Supplemental video 6:
406 <https://youtu.be/Wzn37N3sNDc>). Investigation of the venter of the thorax of *H. “timberlakei*”
407 reveals that, like most *Perdita*, it has a specialized patch of apically hooked hairs where the
408 pollen accumulates (Fig. 4C–D). Finally, in a subsequent review of old videos, I found one of a
409 *Hesperapis* (likely *H. “timberlakei*”) feeding on pollen by first accumulating on the venter, but
410 unfortunately only captured a short and obstructed video (Supplemental video 7:
411 <https://youtu.be/NK10JpnzblI>).

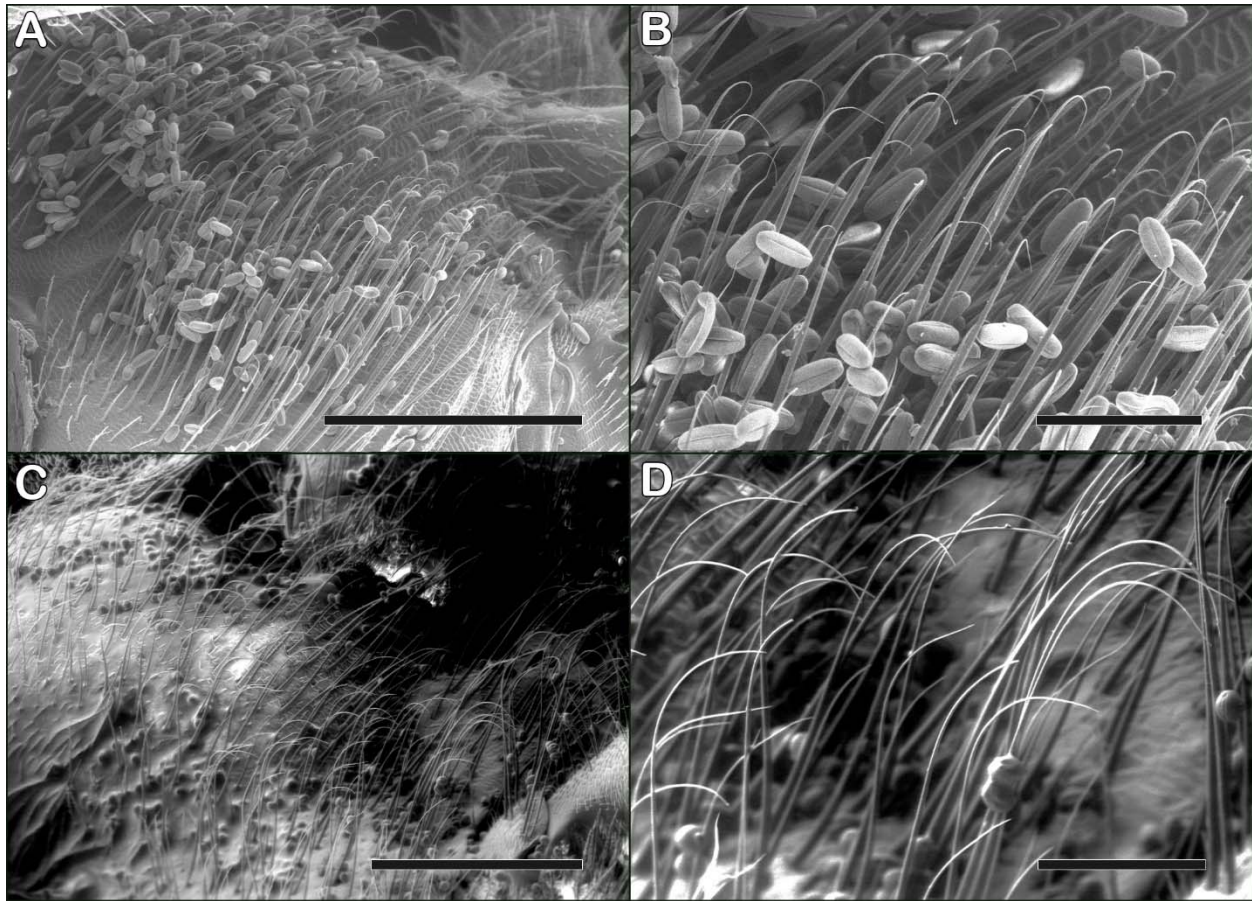
412



413

414 Figure 3. *Hesperapis “timberlakei”* MS gathering pollen A) gathering pollen and accumulating
415 pollen on the venter of the thorax. B) Bringing pollen forward to the mouthparts for moistening
416 whilst in flight; red arrow indicating bright orange *Psorothamnus* pollen on the foreleg. See

417 Supplemental video 5: <https://youtu.be/Tpbd2UrmLIs> and Supplemental video 6:
418 <https://youtu.be/Wzn37N3sNDc>.



419
420 Figure 4. Patch of specialized ventral hairs in *Perdita perpallida* (the ventral patch on *P.*
421 *tortifoliae* is similar) and *Hesperapis* “*timberlakei*” MS. Shown is the venter of the thorax; bees
422 are positioned upside-down and with the head to the left. A) *Perdita perpallida*, scale bar = 400
423 μm . B) *Perdita perpallida* scale bar = 100 μm . C) *Hesperapis* “*timberlakei*” MS, scale bar = 400
424 μm . D) *Hesperapis* “*timberlakei*” MS, scale bar = 100 μm .

425

426 The similarities between the pollen-gathering and pollen-feeding behavior of *H. “timberlakei”*
427 and *P. tortifoliae* are significant because *H. “timberlakei”* is in the family Melittidae, the basal bee
428 family. This supports the hypothesis that gathering pollen by first accumulating it on the venter is
429 basal as well. Unfortunately, due to the relative rarity of melittid bees, observations of their
430 pollen gathering and feeding behavior are frustratingly sparse. One other *Hesperapis* species,
431 *H. laticeps*, has been observed temporarily accumulating pollen on the venter, though the pollen

432 is primarily held in genal hair baskets located on the venter of the head rather than the venter of
433 the thorax (Portman et al. 2019). However, other than the location of the ventral hair patch, the
434 pollen gathering movements are similar between *H. "timberlakei"* and *H. laticeps*, the and the
435 location of the patch on the head is likely an adaptation to extracting pollen from flowers of
436 *Mentzelia* and *Eucnides*. Other melittid bees in the genus *Macropis*, which transport oil-
437 moistened pollen on hind leg scopae, have been found to also gather pollen by accumulating it
438 on the venter before transferring it to the scopae (Cane et al. 1983, Vogel 1992, Schäffler and
439 Dötterl 2011), suggesting this behavior is likely more widespread but unreported in the family.

440 **The evolution of glazed and dry transport from moist pollen transport**

441 The origin of external dry transport likely evolved from moist transport by the stages laid out in
442 Portman and Tepedino (2017). In short, bees that transported moistened pollen underwent an
443 evolutionary transition to dry transport by initially packing pollen dry into the scopae before
444 capping it with moistened pollen. This process was facilitated by bees that switched to host
445 plants with adhesive pollen that stayed in the scopae without the need to be agglutinated by
446 nectar. However, due to the short length of the scopal hairs, only a small amount of pollen could
447 be carried dry, and any additional pollen needed to be agglutinated with nectar on top of the
448 initial layer of dry pollen. Over evolutionary time, the proportion of dry pollen gradually increased
449 as the scopal hairs developed and extended and were able to carry greater amounts of dry
450 pollen. One exception occurs in *Perdita* that utilize Onagraceae pollen — as this pollen has
451 naturally occurring sticky viscin threads that are transported most effectively on sparse, simple
452 scopal hairs (Linsley 1958). The end result of this process was that many bees transitioned to
453 completely dry transport, while other species in the present day retain the vestige of this
454 process and still glaze the pollen, or cap it with moistened pollen. One important point from
455 Portman and Tepedino (2017) is that the evolution of dry transport is associated with
456 specialization on certain pollen types, especially spiky or sticky pollen that either makes moist
457 transport less efficient, dry transport easier, or a combination of both.

458 Glazed pollen transport, where bees initially pack dry pollen into their scopae but then cap it
459 with moistened pollen, appears to be something of a transition state between moist and dry
460 transport (Portman and Tepedino 2017). However, in various species glazed pollen transport
461 appears to be an evolutionary endpoint in and of itself; examples of this include various *Perdita*,
462 *Hesperapis*, and *Dufourea novaeangliae* (Eickwort et al. 1986, Portman and Tepedino 2017).

463 No doubt further investigation will reveal more species that transport glazed pollen. It's unclear
464 why some species continue to glaze pollen rather than evolving entirely dry transport.

465 One important aspect of the evolution of dry pollen transport is that it often leads to the loss of
466 the transition step where bees temporarily accumulating pollen on a specialized hair patch on
467 the venter. However, this behavior is retained in some bees that transport dry pollen. For
468 example, temporarily accumulating pollen on the venter is retained in *Macrotera* subgenus
469 *Macrotera*, which transports dry Cactaceae pollen in tibial scopae (e.g. Neff and Danforth 1991),
470 while the rest of the genus transports moistened pollen. However, many other lineages that
471 have switched to dry pollen transport lose the temporary accumulating pollen step, and instead
472 directly pass pollen to the scopae, or even gather pollen directly with the scopae by rubbing or
473 tapping the scopae directly against the pollen source as in many Megachilidae (Portman et al.
474 2019). The loss of the temporary accumulation of pollen in the pollen gathering process makes
475 the evolutionary transition from moist pollen transport to dry pollen transport a one-way street,
476 since that step is generally necessary to transport moistened pollen.

477 **The further evolution of dry transport and the shifting of the scopal hairs**

478 The transport of dry pollen is associated with the expansion of the scopal hairs to new areas. All
479 bees that transport moistened pollen transport it exclusively on the hind tibia and basitarsus.
480 The greatest degree of scopal expansion in bees that transport moist pollen is that the pollen
481 carrying area has expanded to the rear of the hind tibia and basitarsus, forming a complete
482 “muff” of pollen that encircles the leg (e.g. Malyshev 1936, Rozen 1989). In contrast, the
483 transport of dry or glazed pollen is often associated with the expansion of the scopal hairs to
484 entirely new areas of the body. For example, in some species, the transport of glazed (partially
485 dry) pollen is associated with the expansion of the pollen-transporting hairs to more proximal
486 hind leg segments (e.g. Portman and Tepedino 2017). In terms of broad-scale evolutionary
487 trends in bees, there is a parallel change in different bee groups, with the scopal hairs
488 expanding or migrating from the distal to the proximal areas of the body. This is most clearly
489 demonstrated in *Andrena*, *Colletes*, and various Halictidae, where the majority of pollen is
490 carried on the thorax, sterna, and basal leg segments rather than the hind tibia and basitarsus
491 (Roberts and Vallespir 1978, Michener 1999), which I contend represents the ancestral location
492 of the scopa. Why the scopae have become increasingly proximal is not clear, but it could be an
493 adaptation to better secure the pollen from being scraped off by nesting substrate or forces from
494 wind during flight.

495 The expansion and migration of scopae is particularly intriguing in the evolution of Megachilidae,
496 which transport pollen on abdominal scopa. For most other bee groups, the migration of the
497 scopae is straightforward, with additional scopal areas being added, but relatively minimal loss
498 of pre-existing scopal structures. For example, in some groups, such as *Colletes* and *Andrena*,
499 the scopal hairs of the hind tibia and basitarsus are reduced, but not lost altogether. In most
500 Megachilidae, however, the scopal hairs have moved entirely to the venter of the abdomen
501 without retaining the ancestral scopae. I believe the most likely explanation is that the ancestor
502 to Megachilidae evolved extensive scopal hairs that covered the legs and abdomen (similar to
503 modern-day *Systropha*), and then the scopae was reduced for some unknown reason, leaving
504 only the abdominal scopae. Some basal groups of Megachilidae, such as the genus *Apidosmia*,
505 retain scopal hairs on the hind legs (Gonzalez et al. 2012) and may provide clues as to why
506 other Megachilidae have apparently lost hind leg scopae.

507 Another open question is why some bee groups have not undergone significant scopal
508 expansion despite transporting dry pollen. Examples of this include the genera *Anthophora* and
509 *Xylocopa*, which transport surprisingly small pollen loads primarily on the hind tibia and
510 basitarsis with only a little bit on the hind femur (Roberts and Vallespir 1978). One potential
511 explanation is that they may supplement the external pollen loads with pollen transported
512 internally in the crop. This is thought to occur in *Xylocopa* (Roubik 1989), but whether this also
513 occurs in other groups like *Anthophora* is unknown.

514 **The evolution of oil transport**

515 The evolution of external transport of oil-moistened pollen is unclear and I have not had the
516 opportunity to perform a firsthand investigation of oil-transporting bees. However, some level of
517 inference can still be made about the evolution of this behavior based on known facts. Most
518 importantly, oil-collecting bees also temporarily accumulate pollen on a specialized hair patch
519 on the venter, just like bees that moisten the pollen with nectar. This pollen-accumulating patch
520 appears to be much more extensive in oil-collecting bees; for example, the pollen-accumulating
521 hairs in *Macropis* take up nearly the entire underside of the bee, including the abdominal sterna
522 as well as the venter of the thorax (Cane et al. 1983, Schäffler and Dötterl 2011). The shared
523 behavior of accumulating pollen on the venter suggests that oil-transport may have evolved
524 from bees that originally moistened with nectar. This hypothesis is supported by the findings that
525 the provisions of oil-collecting bees have been found to contain appreciable amounts of sugars.
526 For example, the provisions *Centris maculifrons* contains glucose and fructose in addition to oil

527 (Neff and Simpson 1981), and *C. brethesi* provisions also contained large amounts of sugars
528 (Simpson et al. 1990). However, for these bees, it's not clear if these sugars came from nectar
529 transported in the crop and later added to provisions, versus whether the scopae also transport
530 pollen moistened with some amount of nectar in addition to oil. More information is needed and
531 the evolution of external transport of pollen moistened with oil remains an open question.

532 **The secondary evolution of crop transport**

533 Despite my hypothesis that internal pollen transport is the original form of pollen transport in
534 bees, most if not all known examples of bees that transport pollen internally represent
535 secondary evolutions of this behavior. There have been two instances where this has been
536 examined in-depth: in the genus *Leioproctus* (Houston 1981) and in the *Colletes fasciatus*-group
537 (Kuhlmann 2006). In both cases, the bees evolved from ancestors that transported dry pollen on
538 external scopae (Houston 1981, Kuhlmann 2006). The switch to internal pollen transport is
539 thought to be associated with a switch from hosts with large pollen to small pollen; this switch
540 resulted in scopae that were ill-adapted to carry these fine pollen grains, driving the evolution to
541 internal transport (Houston 1981, Kuhlmann 2006).

542 One intriguing possibility is that internal pollen transport has evolved repeatedly from dry
543 transport because it never quite went away entirely. In other words, at least some bees that
544 transport pollen externally may have never completely lost internal pollen transport and
545 continued to transport a portion of pollen in the crop. Partial internal transport is something that
546 has been mentioned by a couple of authorities on bee behavior and evolution, but to my
547 knowledge it has never been investigated in depth. For example, Roubik (1989) states “A
548 number of nonparasitic bees, for example *Ceratina* and *Xylocopa*, appear to collect pollen in
549 both manners [internally and externally] and display a moderate reduction of scopae.
550 Explanations for this behavior are lacking.” In addition, Michener (2007) cryptically states:
551 “Finally, although pollen in bees’ crops is partly used for their own nutrition, some is carried to
552 the nests and regurgitated.” If crop pollen transport was never completely lost, it would help
553 explain why it has been able to evolve repeatedly in disparate bee lineages.

554 **The secondary evolution of moist transport**

555 The secondary evolution of moist transport from dry transport appears to be rare in bees. While
556 there are some relatively well-documented examples of bees evolving dry or glazed transport
557 from ancestors that transported moistened pollen (e.g. Portman and Tepedino 2017), there are

558 no well-documented examples of the reverse (though it's not clear how hard anyone has
559 looked). This can be explained, at least in part, by the loss of behavior and structures to
560 accumulate pollen on the venter. Most bees that transport dry pollen have lost the specialized
561 patch of hairs to temporarily accumulate pollen and instead pass the pollen directly to the
562 scopae or even gather it directly with the scopae. Without the temporary accumulation step, the
563 behavior used to moisten the pollen before transferring it to the scopae is lost.

564 However, bees can potentially secondarily evolve moist transport if they develop a different
565 behavior to moisten the pollen. This appears to be the case in at least some *Andrena*
566 (*Dactylandrena*) species. For example, within the BBSL collection, there are specimens of
567 *Andrena (Dactylandrena) porterae* that appear to have moistened pollen in the scopae. These
568 bees gather pollen from the inaccessible flowers of *Ribes* using the mouthparts. The act of
569 gathering pollen directly with the mouthparts can provide a mechanism to moisten the pollen
570 with nectar that does not require passing the pollen up to the mouthparts to be moistened, as is
571 done when bees temporarily accumulate pollen on the venter. However, there are also many
572 bees that gather pollen with the mouthparts but still clearly transport dry pollen, so the steps
573 driving the secondary evolution of moist pollen transport are not entirely clear and require more
574 investigation.

575 **Parallel evolution in pollen wasps**

576 In addition to bees, an evolutionary change to provisioning the young with pollen from an
577 ancestral predatory lifestyle has arisen in two other hymenopteran lineages. These examples
578 can inform about how this process occurred in bees. The two examples include the masarid
579 pollen wasps in Vespoidea, and the genus *Krombeinalictus* in Crabronidae. The biology of the
580 single species of *Krombeinalictus* is poorly known, so the lessons that can be learned from it are
581 limited (Krombein and Norden 1997). However, the biology masarid wasps are relatively well-
582 known, and offer a valuable source of information regarding the evolution of pollen provisioning
583 from an ancestral predatory lifestyle.

584 Using masarid pollen wasps (hereafter referred to as “pollen wasps”) as a template, we can
585 compare them to the proposed sequence of bee evolution. This is important because there are
586 many pollen wasps that have a life history similar to the hypothesized protobee. This
587 demonstrates that the proposed stages of bee evolution are not just abstract intellectual
588 constructs, but instead represent viable life-history strategies that exist in the present day.

589 **Hypothesis: Crop transport is ancestral and it evolved from ancestral adult pollen-**
590 **feeding behavior.** All known pollen wasps transport pollen internally, making it clear that it is
591 the ancestral form of pollen transport. Similar to what I have hypothesized for bees, internal
592 transport in pollen wasps is thought to have evolved from ancestral pollen feeding behavior, in
593 this case in stem-group vespid wasps that consumed pollen as adults but provisioned their
594 larvae with prey (Mauss 2007). The antiquity of pollen-feeding behavior in adults is further
595 supported by the ubiquity of this behavior in the present day, where adult pollen wasps of both
596 sexes consume pollen for their own nutritional needs (Mauss et al. 2005, 2019). This is most
597 well-documented in males, of which multiple species have been observed collecting pollen and
598 dissections have found pollen in their crop (Mauss and Müller 2000, 2016, Mauss et al. 2003,
599 2005, 2006, Grodeck et al. 2004). Because females transport pollen internally, it's difficult to
600 determine whether the pollen they consume is for provisions or their own nutrition. However,
601 dissection of female *Pseudomasaris edwardsii* revealed pollen in the mid- and hindgut,
602 confirming that they consumed pollen for their own nutrition (Torchio 1970). These examples
603 suggest that pollen consumption is widespread in adult pollen wasps.

604 **A discussion of the mechanisms by which pollen wasps feed on pollen.** Like bees, pollen
605 wasps gather pollen in two ways, nibbling directly with the mouthparts and by drawing the
606 foreleg through the mouthparts. Nibbling pollen directly with the mouthparts is present in many
607 pollen wasps (Mauss et al. 2019) and likely represents the ancestral form. This type of pollen
608 gathering is most well-documented in *Pseudomasaris edwardsii* (Torchio 1970, Neff and Hook
609 2007), *Quartinia tenerifinia* (Mauss and Mauss 2016), and *Ceramius hispanicus* (Krenn et al.
610 2002). As in bees, nibbling directly with the mouthparts appears to be relatively rare and
611 drawing the forelegs through the mouthparts to consume pollen is the more common form.
612 Indeed, a pollen-comb on the galea has been found in pollen wasps, where it is presumably
613 used to remove pollen from the forelegs as they are drawn through the mouthparts (Krenn et al.
614 2002, Mauss et al. 2019). Multiple species of pollen wasp have been documented to gather
615 pollen through a combination of nibbling with the mandibles and drawing the forelegs through
616 the mouthparts. This is seen in species such as *Celonites fischeri* (Mauss and Müller 2014),
617 *Ceramius fonscolombeii* (Mauss et al. 2003), *Quartinia canariensis* (Mauss and Müller 2016),
618 and *Quartinia major* (Mauss et al. 2018). The use of forelegs in pollen gathering may be related
619 to the accessibility of the pollen; *C. hispanicus* is reported to nibble pollen when anthers are
620 accessible, and uses the forelegs when they are not (Mauss and Müller 2000, Krenn et al.
621 2002).

622 **Temporary accumulation of pollen in pollen wasps.** Similar to bees, many species of pollen
623 wasps also temporarily accumulate pollen, with the pollen initially gathered onto a specialized
624 patch of hairs before being brought to the mouthparts by the forelegs (Müller 1996). The most
625 well-documented examples of the temporary accumulation of pollen in wasps include species
626 that first accumulate pollen on the face, often on knobbed or hooked hairs (Müller 1996, Mauss
627 2006, Mauss et al. 2016). Other pollen wasps accumulate pollen on the dorsum of the thorax via
628 “rasping” behavior (Torchio 1974, Portman et al. 2019). Most importantly, there are pollen wasp
629 species that gather pollen by first accumulating pollen on the venter of the thorax. For example,
630 *Rolandia maculata* has a specialized patch of stiff hairs with bent tips on the venter of the
631 thorax; this patch accumulates pollen before being ingested using the forelegs (Houston 1995).
632 A similar pollen-accumulating hair patch is found on the venter of *Ceramius braunsi* (Gess and
633 Gess 1989). Although Gess and Gess (1989) describe the pollen gathering in *C. braunsi* as
634 being performed solely by the forelegs, without an accumulation step, the accumulation of
635 pollen in the ventral hair patches suggests Gess and Gess (1989) may have missed that
636 behavior.

637 **Tying back to bees.** Although there are no pollen wasps that are known to transport pollen
638 externally, there are still important parallels to the hypothesized evolution of pollen transport in
639 bees. Specifically, in both bees and pollen wasps, adults feed on pollen for their own nutritional
640 needs and they can consume pollen either through nibbling or drawing the foreleg through the
641 mouthparts. Importantly, crop transport of pollen is unambiguously ancestral in pollen wasps,
642 and some pollen wasps share the behavior of temporarily accumulating pollen on the venter. It
643 is especially striking that there are pollen wasps that gather and transport pollen the same way
644 that *Perdita tortifoliae* gathers and consumes pollen, which lends credence to the hypothesis
645 that temporarily accumulating pollen on the venter (as exemplified by *Perdita tortifoliae* in earlier
646 sections) represents an ancestral form of pollen transport in bees. However, masarids have
647 clearly never made the evolutionary transition to external transport. The lack of this evolutionary
648 innovation could help explain why bees are so much more diverse than masarids, despite their
649 similar evolutionary ages. Overall, this supports the hypothesis that bees and masarids followed
650 a similar evolutionary pathway in the initial stages of the evolution of pollen transport.

651 **Conclusion**

652 In this paper I have laid out a hypothesis on the origin and evolution of pollen transport in bees.
653 Under this view, internal transport in the crop represents the original pollen transport behavior

654 and it evolved from pollen feeding in adults. From there, bees evolved the ability to temporarily
655 accumulate pollen on a specialized patch on the venter of the thorax, which represents a
656 necessary transition stage that led to external transport of pollen moistened with nectar on the
657 hind legs. External transport of dry or glazed pollen then evolved from external moist transport.
658 Finally, the evolution of external dry pollen transport led to the expansion of the scopal hairs in
659 many bee groups. This hypothesis is supported by multiple lines of evidence, particularly by
660 observations on present-day pollen-feeding and pollen-gathering behavior in bees which allow
661 us to reconstruct the evolutionary history of these behaviors. Importantly, comparing the
662 evolution of pollen transport of bees and pollen wasps boosts this hypothesis because it
663 highlights potential paths of parallel evolution and demonstrates that the hypothesized transition
664 forms in bees are actually viable life history strategies that exist in the present day in some
665 pollen wasps.

666 Under the hypothesis laid out here, the evolution of external pollen transport in bees can be
667 reconstructed by examining the steps of present-day pollen gathering behavior. In the present
668 day, the transport of moistened pollen requires a transition step (temporary accumulation of
669 pollen on the venter) that results in pollen taking a complicated and circuitous route: first the
670 pollen is picked up by the forelegs, then transferred to a temporary holding area on the venter of
671 the thorax, this pollen is then picked back up by the forelegs, brought forward to the mouthparts
672 where it is moistened with nectar, passed backwards again where it is scraped off the forelegs
673 by the midlegs before finally being deposited onto the hind legs. However, this process can be
674 explained if it is viewed as the result of external moist pollen transport evolving by simply adding
675 additional behaviors onto internal pollen transport; in moist transport, the original behavior of
676 bringing pollen forward to be consumed by the mouthparts is retained, but instead of being
677 consumed, the pollen is instead moistened and passed back the hindlegs. Most importantly,
678 each individual stage of this evolutionary process is adaptive in its own right. The consumption
679 of pollen via the foreleg and the temporary accumulation of pollen are both behaviors that are
680 seen in the present day in both bees and pollen wasps.

681 My hypothesis that internal pollen transport is ancestral in bees marks a return to the earliest
682 hypotheses regarding the genesis of bees, which was first laid out by Müller (1883) and
683 expanded by Malyshev (1969) and Jander (1976). All of the previous workers cited the hairless
684 bodies, poorly-developed pollen brushes, short tongues, and similarity to sphecid wasps as
685 evidence that *Hylaeus* represented an ancestral bee group. Although recent molecular
686 phylogenies have made it clear that *Hylaeus* and other Colletidae are not basal (Danforth et al.

687 2012), it does not negate the fact that the protobee almost certainly did have many of those
688 characteristics, particularly poorly-developed body hairs and pollen-collecting structures and
689 behaviors. In other words, the fact that *Hylaeus* are not basal does not invalidate the other
690 logical arguments in favor of crop transport being ancestral. In particular, the parallel evolution
691 with pollen wasps is one of the strongest arguments in favor of crop transport being ancestral,
692 which is further bolstered by the degree of similarity in their evolutionary development laid out in
693 the previous section.

694 Under this framework, I contend that moist pollen transport is ancestral to dry pollen transport.
695 This represents the first detailed hypothesis about how moist pollen transport could have
696 evolved (with perhaps the exception of Michener et al. (1978)), and it marks a deviation from the
697 conventional wisdom that moist transport evolved from dry transport (Müller 1883, Michener
698 1944, Michener et al. 1978, Roberts and Vallespir 1979, Pasteels et al. 1983). The assumption
699 that moist transport is the more derived character seems to stem, at least in part, by the notion
700 that Apidae, and especially honeybees, represent the most advanced or “most derived” bees
701 (e.g. Müller 1883, Jander 1976, Michener 1979). The hypothesis that moist transport represents
702 the ancestral form of external pollen transport makes sense because it does not require
703 specialized morphological characters such as well-developed branched hairs or scopae. Indeed,
704 it allows pollen types of a wide variety of sizes and shapes to be carried on short and sparse
705 simple hairs instead of the scopal adaptations typically seen in bees that transport dry pollen
706 (Roberts and Vallespir 1978, Portman and Tepedino 2017, Danforth et al. 2019). In contrast, the
707 evolution of dry transport from moist transport is associated with the elaboration, specialization,
708 and expansion of the scopal hairs (Portman and Tepedino 2017).

709 Most importantly, the hypotheses laid out here create a consistent framework that is informed by
710 present-day bee behavior and allows us to make broad predictions about the biology and
711 evolution of bees. The most important of these predictions are laid out below:

- 712 1. Additional studies on Melittidae and other basal bees will reveal that most groups
713 transport moistened pollen.
- 714 2. Most bees that transport moistened pollen temporarily accumulate pollen on the venter
715 (or gather pollen directly with mouthparts). The obvious exceptions here are *Apis* and
716 *Bombus* (but not *Trigona s.l.*, see Michener et al 1978); it’s not clear why this is the case
717 but this should be a derived condition.

- 718 3. Additional studies will also reveal that species that gather pollen by accumulating pollen
719 on a patch of specialized hairs on the venter also accumulate on that patch when
720 feeding on pollen.
- 721 4. In bee lineages where there has been a transition between moist and dry external
722 transport, moist transport will be found to be ancestral (except when pollen is gathered
723 directly with the mouthparts).
- 724 5. The evolutionary transition of moist transport to dry transport will be associated with the
725 use of pollen that is particularly adhesive, large, or spiny, which would make them more
726 efficiently transported dry (e.g. Portman and Tepedino 2017).
- 727 6. Investigation of bees that transport pollen externally will reveal bees that transport a
728 portion of pollen internally as well. This is particularly relevant for Melittidae and bees
729 with small scopae.
- 730 7. Additional studies of the pollen gathering behavior of pollen wasps will reveal species
731 that gather pollen by temporarily accumulating it on the venter before transferring to the
732 mouthparts (as in Houston 1995).
- 733 8. The broad and flattened hind basitarsus, a character shared by all bees that separates
734 them from wasps (Radchenko and Pasenko 1996, Engel 2001, Michener 2007), is a
735 result of that being the location of the original external scopae. This suggests that the
736 most recent common ancestor of all extant bees transported external moist pollen on the
737 hind legs.
- 738 9. Evolutionary trends will reveal that bees have undergone an expansion of the area of
739 scopal hairs from the ancestral location on the hind tibia and basitarsus (rather than the
740 reverse — a consolidation onto the hind tibia and basitarsus).

741 The last prediction stands in strong contrast to the primary competing hypothesis regarding the
742 origin of pollen transport, originally proposed by Radchenko and Pasenko (1996) and supported
743 by Michener (2007). Under that hypothesis, external dry transport is ancestral and scopal hairs
744 coalesced and specialized from a diffuse and unspecialized ancestral form. This creates a key
745 difference between their hypothesis and my own. Under my hypothesis, where moist transport is
746 ancestral to dry transport, the hind tibia and basitarsus are the ancestral location of the scopa,
747 and all modern-day external scopae have expanded outward from there. In contrast, under the
748 hypothesis of Radchenko and Pasenko (1996), the reverse would be predicted — that diffuse

749 scopae should coalesce on the hind tibia and basitarsus. My prediction that scopae that
750 transport dry pollen will have become increasingly proximal rather than increasingly distal also
751 stands in contrast to the conventional wisdom regarding the evolution of pollen transport
752 (Pasteels and Pasteels 1979, Thorp 1979, Pasteels et al. 1983, Westerkamp 1996). Based on
753 the evidence currently available (e.g. Roberts and Vallespir 1978), expansion of the scopae,
754 rather than the consolidation, appears to be the rule, though this has yet to be rigorously tested
755 from a phylogenetic standpoint.

756 Here, I have presented the first detailed hypothesis of how external moist transport could have
757 evolved and this marks a step forward in a field that has seen little progress despite the major
758 advances in our understanding of bee phylogenies and deep evolutionary relationships. Further,
759 this framework allows for us to better understand bee biology in the present day, and offers an
760 evolutionary explanation for behaviors, such as the temporary accumulation of pollen on the
761 venter, that may at first seem incongruous. It is my hope that this will stimulate the research
762 needed to confirm or refute this hypothesis. While better-resolved phylogenies would certainly
763 be helpful, answers about the origin and evolution of pollen transport primarily require studies
764 on the natural history, behavior, and functional morphology of bees and related Hymenoptera.

765

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771 **References**

772 Batra SWT (1964) Behavior of the social bee, *Lasioglossum zephyrum*, within the nest
773 (Hymenoptera: Halictidae). *Insectes Sociaux* 11: 159–185.

774 <https://doi.org/10.1007/BF02222935>

775 Batra SWT (1985) Red maple (*Acer rubrum* L.), an important early spring food resource for
776 honey bees and other insects. *Journal of the Kansas Entomological Society* 58: 169–172.

- 777 Bohart GE, Menke AS (1976) Sphecid Wasps of the World. University of California Press, 695
778 pp.
- 779 Bohart RM, Grissell EE (1972) Nesting habits and larva of *Pulverro monticola*. Pan-Pacific
780 Entomologist 48: 145–149.
- 781 Branstetter MG, Danforth BN, Pitts JP, Faircloth BC, Ward PS, Buffington ML, Gates MW, Kula
782 RR, Brady SG (2017) Phylogenomic insights into the evolution of stinging wasps and the
783 origins of ants and bees. Current Biology 27: 1019–1025.
784 <https://doi.org/10.1016/j.cub.2017.03.027>
- 785 Cane JH (2016) Adult pollen diet essential for egg maturation by a solitary *Osmia* bee. Journal
786 of Insect Physiology 95: 105–109. <https://doi.org/10.1016/j.jinsphys.2016.09.011>
- 787 Cane JH, Dobson HEM, Boyer B (2016) Timing and size of daily pollen meals eaten by adult
788 females of a solitary bee (*Nomia melanderi*) (Apiformes: Halictidae). Apidologie 48: 17–30.
789 <https://doi.org/10.1007/s13592-016-0444-8>
- 790 Cane JH, Eickwort GC, Wesley FR, Spielholz J (1983) Foraging, grooming and mate-seeking
791 behaviors of *Macropis nuda* (Hymenoptera, Melittidae) and use of *Lysimachia ciliata*
792 (Primulaceae) oils in larval provisions and cell linings. American Midland Naturalist 110:
793 257–264. <https://doi.org/10.2307/2425267>
- 794 Cardinal S, Danforth B (2013) Bees diversified in the age of eudicots. Proceedings of the Royal
795 Society B Biological Sciences 280: 20122686.
796 <https://doi.org/http://dx.doi.org/10.1098/rspb.2012.2686>
- 797 Carpenter JM (2001) Checklist of species of the subfamily Masarinae (Hymenoptera: Vespidae).
798 American Museum Novitates 3325: 1–40. [https://doi.org/10.1206/0003-
799 0082\(2001\)325<0001:COOTS>2.0.CO;2](https://doi.org/10.1206/0003-0082(2001)325<0001:COOTS>2.0.CO;2)
- 800 Danforth BN (1989) Nesting behavior of four species of *Perdita* (Hymenoptera: Andrenidae).
801 Journal of the Kansas Entomological Society 62: 59–79. Available from:
802 <http://www.jstor.org/stable/10.2307/25085052>.
- 803 Danforth BN (1990) Provisioning behavior and the estimation of investment ratios in a solitary
804 bee, *Calliopsis (Hypomacrotera) persimilis* (Cockerell) (Hymenoptera: Andrenidae).

- 805 Behavioral Ecology and Sociobiology 27: 159–168. <https://doi.org/10.1007/BF00180299>
- 806 Danforth BN (1996) Phylogenetic analysis and taxonomic *Macrotera*, *Macroteropsis*,
807 *Macroterella* and *Cockerellula* (Hymenoptera: Andrenidae). University of Kansas Science
808 Bulletin 55: 635–692. <https://doi.org/citeulike-article-id:1327877>
- 809 Danforth BN, Minckley RL, Neff JL (2019) The solitary bees: Biology, evolution, conservation.
810 Princeton University Press, Princeton and Oxford, 472 pp.
811 <https://doi.org/10.2307/j.ctvd1c929>
- 812 Danforth BN, Sipes S, Fang J, Brady SG (2006) The history of early bee diversification based
813 on five genes plus morphology. Proceedings of the National Academy of Sciences 103:
814 15118–15123. <https://doi.org/10.1073/pnas.0604033103>
- 815 Danforth BN, Cardinal S, Praz C, Almeida E a B, Michez D (2012) The impact of molecular data
816 on our understanding of bee phylogeny and evolution. Annual Review of Entomology 58:
817 57–78. <https://doi.org/10.1146/annurev-ento-120811-153633>
- 818 Debevec AH, Cardinal S, Danforth BN (2012) Identifying the sister group to the bees: a
819 molecular phylogeny of Aculeata with an emphasis on the superfamily Apoidea. Zoologica
820 Scripta 41: 527–535. <https://doi.org/10.1111/j.1463-6409.2012.00549.x>
- 821 Eickwort G, Kukuk P, Wesley F (1986) The nesting biology of *Dufourea novaeangliae*
822 (Hymenoptera: Halictidae) and the systematic position of the Dufoureae based on
823 behavior and development. Journal of the Kansas Entomological Society 59: 103–120.
824 Available from: <http://www.jstor.org/stable/25084743> (June 3, 2014).
- 825 Engel MS (2001) A monograph of the Baltic Amber bees and evolution of the Apoidea
826 (Hymenoptera). Bulletin of the American Museum of Natural History 259: 1–192.
827 [https://doi.org/10.1206/0003-0090\(2001\)259<0001:AMOTBA>2.0.CO;2](https://doi.org/10.1206/0003-0090(2001)259<0001:AMOTBA>2.0.CO;2)
- 828 Farish DJ (1972) The evolutionary implications of qualitative variation in the grooming behaviour
829 of the Hymenoptera (Insecta). Animal behaviour 20: 662–76. Available from:
830 <http://www.ncbi.nlm.nih.gov/pubmed/4661314>.
- 831 Gess SK, Gess FW (1989) Flower visiting by masarid wasps in southern Africa (Hymenoptera:
832 Vespoidea: Masaridae). Annals of the Cape provincial Museums 18: 95–134. Available

- 833 from: <https://www.biodiversitylibrary.org/item/215761>.
- 834 Gonzalez VH, Griswold T, Praz CJ, Danforth BN (2012) Phylogeny of the bee family
835 Megachilidae (Hymenoptera: Apoidea) based on adult morphology. Systematic
836 Entomology 37: 261–286. <https://doi.org/10.1111/j.1365-3113.2012.00620.x>
- 837 Grimaldi DA, Peñalver E, Barrón E, Herhold HW, Engel MS (2019) Direct evidence for eudicot
838 pollen-feeding in a Cretaceous stinging wasp (Angiospermae; Hymenoptera, Aculeata)
839 preserved in Burmese amber. Communications Biology 2: 1–10.
840 <https://doi.org/10.1038/s42003-019-0652-7>
- 841 Groddeck J, Mauss V, Reinhold K (2004) The resource-based mating system of the
842 mediterranean pollen wasp *Ceramius fonscolombei* Latreille 1810 (Hymenoptera,
843 Vespidae, Masarinae). Journal of Insect Behavior 17: 397–418.
844 <https://doi.org/10.1023/B:JOIR.0000031539.52983.89>
- 845 Houston T (2019) A guide to native bees of Australia. CSIRO Publishing, 280 pp.
- 846 Houston TF (1981) Alimentary transport of pollen in a paracolletine bee (Hymenoptera:
847 Colletidae). Australian Entomological Magazine 7: 57–59.
- 848 Houston TF (1995) Notes on the ethology of *Rolandia maculata* (Hymenoptera: Vespidae:
849 Masarinae), a pollen wasp with a psammophore. Records of the Western Australia
850 Museum 17: 343–349.
- 851 Hunt JH, Brown PA, Sago KM, Kerker JA (1991) Vespid wasps eat pollen (Hymenoptera:
852 Vespidae). Journal of the Kansas Entomological Society 64: 127–130. Available from:
853 <http://www.jstor.org/stable/25085261>.
- 854 Jander R (1976) Grooming and pollen manipulation in bees (Apoidea): the nature and evolution
855 of movements involving the foreleg. Physiological Entomology 1: 179–194.
856 <https://doi.org/10.1111/j.1365-3032.1976.tb00960.x>
- 857 Jervis MA (1998) Functional and evolutionary aspects of mouthpart structure in parasitoid
858 wasps. Biological Journal of the Linnean Society 63: 461–493.
859 <https://doi.org/10.1111/j.1095-8312.1998.tb00326.x>
- 860 Krenn HW, Mauss V, Plant J (2002) Evolution of the suctorial proboscis in pollen wasps

- 861 (Masarinae, Vespidae). *Arthropod Structure and Development* 31: 103–120.
862 [https://doi.org/10.1016/S1467-8039\(02\)00025-7](https://doi.org/10.1016/S1467-8039(02)00025-7)
- 863 Krombein K V, Norden BB (1997) Nesting behavior of *Krombeinictus nordenae* Leclercq, a
864 sphecid wasp with vegetarian larvae (Hymenoptera, Sphecidae, Crabroninae).
865 *Proceedings of the Entomological Society of Washington* 99: 42–49.
- 866 Kuhlmann M (2006) Scopa reduction and pollen collecting of bees of the *Colletes fasciatus*-
867 group in the winter rainfall area of South Africa (Hymenoptera: Colletidae). *Journal of the*
868 *Kansas Entomological Society* 79: 165–175. <https://doi.org/10.2317/0505.10.1>
- 869 Lanham UN (1980) Evolutionary origin of bees (Hymenoptera: Apoidea). *Journal of the New*
870 *York Entomological Society* 88: 199–209.
- 871 Liebig J, Heinze J, Holldobler B (1997) Trophallaxis and aggression in the ponerine ant, *Ponera*
872 *coarctata*: Implications for the evolution of liquid food exchange in the Hymenoptera.
873 *Ethology* 103: 707–722. <https://doi.org/10.1111/j.1439-0310.1997.tb00180.x>
- 874 Linsley E (1958) The ecology of solitary bees. *Hilgardia* 27: 543–585.
- 875 Malyshev SI (1936) The nesting habits of solitary bees. A comparative study. *Revista Espanola*
876 *de Entomologia* 11: 201–309.
- 877 Malyshev SI (1969) *Genesis of the Hymenoptera and the phases of their evolution*. Springer
878 US, 320 pp. <https://doi.org/10.1007/978-1-4684-7161-8>
- 879 Maneval H (1939) Notes sur les Hymenopteres. *Annales de la Societe Entomologique de*
880 *France* 108: 49–108.
- 881 Mauss V (2006) Observations on flower association and mating behaviour of the pollen wasp
882 species *Celonites abbreviatus* (Villers, 1789) in Greece (Hymenoptera: Vespidae,
883 Masarinae). *Journal of Hymenoptera Research* 15: 266–269.
- 884 Mauss V (2007) Evolution verschiedener Lebensformtypen innerhalb basaler Teilgruppen der
885 Faltenwespen (Hymenoptera, Vespidae). *Denisia* 66: 701–722.
- 886 Mauss V, Müller A (2000) A study of the bionomy of the Spanish pollen wasp *Ceramius*
887 *hispanicus* Dusmet (Hymenoptera, Vespidae, Masarinae): Nesting, mating, and flower

- 888 associations. Journal of Hymenoptera Research 9: 1–17. Available from:
889 <http://biostor.org/reference/251>.
- 890 Mauss V, Müller A (2014) First contribution to the bionomics of the pollen wasp *Celonites*
891 *fischeri* Spinola, 1838 (Hymenoptera, Vespidae, Masarinae) in Cyprus. Journal of
892 Hymenoptera Research 39: 119–153. <https://doi.org/10.3897/JHR.39.7841>
- 893 Mauss V, Müller A (2016) Contribution to the bionomics of the pollen wasp *Quartinia canariensis*
894 Blüthgen, 1958 (Hymenoptera, Vespidae, Masarinae) in Fuerteventura (Canary Islands,
895 Spain). Journal of Hymenoptera Research 50: 1–24. <https://doi.org/10.3897/JHR.50.6870>
- 896 Mauss V, Mauss H (2016) Field observations of the behaviour of the pollen wasp *Quartinia*
897 *tenerifina* Richards, 1969 (Hymenoptera, Vespidae, Masarinae) at flowers in Tenerife
898 (Canary Islands, Spain). Linzer biologische Beiträge 48: 1327–1332.
- 899 Mauss V, Andreas M, Yildirim E (2003) Nesting and flower associations of the pollen wasp
900 *Ceramius fonscolombei* Latreille, 1810 (Hymenoptera: Vespidae: Masarinae) in Spain.
901 Journal of the Kansas Entomological Society 76: 1–15.
- 902 Mauss V, Müller A, Yildirim E (2005) First contribution to the bionomics of the pollen wasp
903 *Ceramius caucasicus*. Entomological Science 13: 42–59. [https://doi.org/10.1111/j.1479-](https://doi.org/10.1111/j.1479-8298.2010.00370.x)
904 [8298.2010.00370.x](https://doi.org/10.1111/j.1479-8298.2010.00370.x)
- 905 Mauss V, Müller A, Prosi R (2006) Mating, nesting and flower association of the east
906 Mediterranean pollen wasp *Ceramius bureschi* in Greece (Hymenoptera: Vespidae:
907 Masarinae). Entomologia Generalis 29: 1–26. <https://doi.org/10.1127/entom.gen/29/2006/1>
- 908 Mauss V, Fateryga A, Prosi R (2016) Taxonomy, distribution and bionomics of *Celonites*
909 *tauricus* Kostylev, 1935, stat. n. (Hymenoptera, Vespidae, Masarinae). Journal of
910 Hymenoptera Research 48: 33–66. <https://doi.org/10.3897/JHR.48.6884>
- 911 Mauss V, Müller A, Prosi R (2018) Flower associations and nesting of the pollen wasp *Quartinia*
912 *major* Kohl, 1898 (Hymenoptera, Masarinae) in Morocco. Journal of Hymenoptera
913 Research 31: 15–31. <https://doi.org/10.3897/jhr.62.22879>
- 914 Mauss V, Kuba K, Krenn HW (2019) Evolution of the multifunctional mouthparts of adult
915 Vespidae. In: Krenn HW (Ed.), Insect Mouthparts: Form, Function, Development and

- 916 Performance. Springer, Cham, Switzerland, 443–478. [https://doi.org/10.1007/978-3-030-](https://doi.org/10.1007/978-3-030-29654-4_14)
917 29654-4_14
- 918 Michener CD (1944) Comparative external morphology, phylogeny, and a classification of the
919 bees (Hymenoptera). *Bulletin of the American Museum of Natural History* 82: 151–326.
- 920 Michener CD (1965) A classification of the bees of the Australian and South Pacific regions.
921 *Bulletin of the American Museum of Natural History* 130: 1–362.
922 <https://doi.org/10.1086/405376>
- 923 Michener CD (1979) Biogeography of the bees. *Annals of the Missouri Botanical Garden* 66:
924 277–347. <https://doi.org/10.3417/2007065>
- 925 Michener CD (1999) The corbiculae of bees. *Apidologie* 30: 67–74.
- 926 Michener CD (2000) What was the protobee? *Anais do IV Encontro sobre Abelhas*: 2–8.
- 927 Michener CD (2007) *The bees of the world*. 2nd ed. Johns Hopkins University Press, Baltimore,
928 953 pp.
- 929 Michener CD, Winston ML, Jander R (1978) Pollen manipulation and related activities and
930 structures in bees of the family Apidae. *University of Kansas Science Bulletin* 51: 575–601.
- 931 Michez D, Vanderplanck M, Engel MS (2012) Fossil bees and their plant associates. In: Patiny
932 S (Ed.), *Evolution of plant-pollinator relationships*. Cambridge University Press, Université
933 de Mons-Hainaut, Belgium, 103–164.
- 934 Michez D, Patiny S, Rasmont P, Timmermann K, Vereecken NJ (2008) Phylogeny and host-
935 plant evolution in *Melittidae s.l.* (Hymenoptera: Apoidea). *Apidologie* 39: 146–162.
936 <https://doi.org/10.1051/apido>
- 937 Müller A (1996) Convergent evolution of morphological specializations in Central European bee
938 and honey wasp species as an adaptation to the uptake of pollen from nototribic flowers
939 (Hymenoptera, Apoidea and Masaridae). *Biological Journal of the Linnean Society* 57:
940 235–252. <https://doi.org/10.1111/j.1095-8312.1996.tb00311.x>
- 941 Müller H (1883) *The fertilisation of flowers*. R. Clay, Sons, and Taylor, London.

- 942 Murray EA, Bossert S, Danforth BN (2018) Pollinivory and the diversification dynamics of bees.
943 Biology Letters 14: 20180530. <https://doi.org/10.1098/rsbl.2018.0530>
- 944 Neff JL, Simpson BB (1981) Oil-collecting structures in the Anthophoridae (Hymenoptera):
945 morphology, function, and use in systematics. Journal of the Kansas Entomological Society
946 54: 95–123.
- 947 Neff JL, Danforth BN (1991) The nesting and foraging behavior of *Perdita texana* (Cresson)
948 (Hymenoptera: Andrenidae). Journal of the Kansas Entomological Society 64: 394–405.
949 Available from: <https://www.jstor.org/stable/25085306>.
- 950 Neff JL, Hook AW (2007) Multivoltinism and usage of multiple nest substrates in a West Texas
951 sand dune population of *Pseudomasaris phacelliae* Rohwer (Hymenoptera: Vespidae:
952 Masarinae). Journal of Hymenoptera Research 16: 266–276.
953 <https://doi.org/10.5860/choice.48-6651>
- 954 Pasteels JJ, Pasteels JM (1979) Etude au microscope electronique a balayage des scopas
955 collectrices de pollen chez les Andrenidae (Hymenoptera: Apoidea: Andrenidae). Archives
956 de Biologie 90: 113–130.
- 957 Pasteels JM, Pasteels JJ, De Vos L (1983) Etude au microscope electronique a balayage des
958 scopas collectrices de pollen chez les Panurginae (Hymenoptera, Apoidea, Andrenidae).
959 Archives de Biologie 94: 53–73.
- 960 Peters RS, Krogmann L, Mayer C, Donath A, Gunkel S, Meusemann K, Kozlov A,
961 Podsiadlowski L, Petersen M, Lanfear R, Diez PA, Heraty J, Kjer KM, Klopstein S, Meier
962 R, Polidori C, Schmitt T, Liu S, Zhou X, Wappler T, Rust J, Misof B, Niehuis O (2017)
963 Evolutionary history of the Hymenoptera. Current Biology 27: 1013–1018.
964 <https://doi.org/10.1016/j.cub.2017.01.027>
- 965 Portman ZM, Tepedino VJ (2017) Convergent evolution of pollen transport mode in two distantly
966 related bee genera (Hymenoptera: Andrenidae and Melittidae). Apidologie 48: 461–472.
967 <https://doi.org/10.1007/s13592-016-0489-8>
- 968 Portman ZM, Orr MC, Griswold T (2019) A review and updated classification of pollen gathering
969 behavior in bees (Hymenoptera, Apoidea). Journal of Hymenoptera Research 71: 171–
970 208. <https://doi.org/10.3897/jhr.71.32671>

- 971 Portman ZM, Ascher JS, Cariveau DP (In Press) Nectar concentrating behavior in bees
972 (Hymenoptera: Anthophila). *Apidologie*.
- 973 Radchenko VG, Pesenko YA (1996) Protobee and its nests: A new hypothesis concerning the
974 early evolution of Apoidea. *Entomological Review* 75: 913–933.
- 975 Richards KW (1994) Ovarian development in the alfalfa leafcutter bee, *Megachile rotundata*.
976 *Journal of Apicultural Research* 33: 199–203.
977 <https://doi.org/10.1080/00218839.1994.11100871>
- 978 Roberts RB, Vallespir SR (1978) Specialization of hairs bearing pollen and oil on the legs of
979 bees (Apoidea: Hymenoptera). *Annals of the Entomological Society of America* 71: 619–
980 627. <https://doi.org/10.1093/aesa/71.4.619>
- 981 Robertson C (1929) *Flowers and insects lists of visitors of four hundred and fifty-three flowers*.
982 Science Press Printing Company, Lancaster, PA, 221 pp.
- 983 Roubik DW (1989) *Ecology and natural history of tropical bees*. Cambridge University Press,
984 Cambridge, 514 pp. [https://doi.org/10.1016/0169-5347\(90\)90188-J](https://doi.org/10.1016/0169-5347(90)90188-J)
- 985 Rozen JG (1958) Monographic study of the genus *Nomadopsis* Ashmead (Hymenoptera:
986 Andrenidae). *University of California Publications in Entomology* 15: 1–202.
- 987 Rozen JG (1989) Life history studies of the primitive Panurgine bees (Hymenoptera:
988 Andrenidae: Panurginae). *American Museum Novitates* 2962: 1–27.
- 989 Sann M, Niehuis O, Peters RS, Mayer C, Kozlov A, Podsiadlowski L, Bank S, Meusemann K,
990 Misof B, Bleidorn C, Ohl M (2018) Phylogenomic analysis of Apoidea sheds new light on
991 the sister group of bees. *BMC Evolutionary Biology* 18: 1–15.
992 <https://doi.org/10.1186/s12862-018-1155-8>
- 993 Sann M, Meusemann K, Niehuis O, Escalona HE, Mokrousov M, Ohl M, Pauli T, Schmid-Egger
994 C (2021) Reanalysis of the apoid wasp phylogeny with additional taxa and sequence data
995 confirms the placement of Ammoplanidae as sister to bees. *Systematic Entomology* 46:
996 558–569. <https://doi.org/10.1111/syen.12475>
- 997 Schäffler I, Dötterl S (2011) A day in the life of an oil bee: phenology, nesting, and foraging
998 behavior. *Apidologie* 42: 409–424. <https://doi.org/10.1007/s13592-011-0010-3>

- 999 Sedivy C, Praz CJ, Müller A, Widmer A, Dorn S (2008) Patterns of host-plant choice in bees of
1000 the genus *Chelostoma*: the constraint hypothesis of host-range evolution in bees.
1001 Evolution; international journal of organic evolution 62: 2487–507.
1002 <https://doi.org/10.1111/j.1558-5646.2008.00465.x>
- 1003 Shinn AF (1967) A revision of the bee genus *Calliopsis* and the biology and ecology of *C.*
1004 *andreniformis* (Hymenoptera, Andrenidae). University of Kansas Science Bulletin 46: 753–
1005 936.
- 1006 Simpson BB, Neff JL, Dieringer G (1990) The production of floral oils by *Monttea*
1007 (Scrophulariaceae) and the function of tarsal pads in Centris bees. Plant Systematics and
1008 Evolution 173: 209–222. <https://doi.org/10.1007/BF00940864>
- 1009 Stage GI (1966) Biology and systematics of the American species of the genus *Hesperapis*
1010 Cockerell. PhD dissertation. University of California, Berkeley
- 1011 Stockhammer KA (1966) Nesting habits and life cycle of a sweat bee, *Augochlora pura*
1012 (Hymenoptera: Halictidae). Journal of the Kansas Entomological Society 39: 157–192.
- 1013 Thorp RW (1979) Structural, behavioral, and physiological adaptations of bees (Apoidea) for
1014 collecting pollen. Annals of the Missouri Botanical Garden 66: 788–812.
1015 <https://doi.org/10.2307/2398919>
- 1016 Timberlake PH (1968) A revisional study of the bees of the genus *Perdita* F. Smith, with special
1017 reference to the fauna of the Pacific Coast (Hymenoptera, Andrenidae). Part VII. University
1018 of California Publications in Entomology 49: 1–196.
- 1019 Torchio PF (1970) The ethology of the wasp, *Pseudomasaris edwardsii* (Cresson), and a
1020 description of its immature forms (Hymenoptera: Vespoidea, Masaridae). Contributed
1021 Science, Los Angeles County Museum of Natural History 202: 1–32.
- 1022 Torchio PF (1974) Mechanisms involved in the pollination of *Penstemon* visited by the masarid
1023 wasp, *Pseudomasaris vespoidea* (Cresson). Pan-Pacific Entomologist 50: 226–234.
- 1024 Visscher PK, Danforth BN (1993) Biology of *Calliopsis pugionis* (Hymenoptera, Andrenidae) -
1025 Nesting, foraging, and investment sex ratio. Annals of the Entomological Society of
1026 America 86: 822–832.

- 1027 Vogel S (1992) Ölblumen und ölsammelnde Bienen [Oil Flowers and Oil-collecting Bees]. IWF
1028 (Göttingen) <https://doi.org/10.3203/IWF/Z-7083>
- 1029 Westerkamp C (1996) Pollen in bee-flower relations: Some considerations on melittophily.
1030 *Botanica Acta* 109: 325–332. <https://doi.org/10.1111/j.1438-8677.1996.tb00580.x>
- 1031 Zheng B, Cao L, Tang P, van Achterberg K, Hoffmann AA, Chen H-Y, Chen X, Wei S (2018)
1032 Gene arrangement and sequence of mitochondrial genomes yield insights into the
1033 phylogeny and evolution of bees and sphecid wasps (Hymenoptera: Apoidea). *Molecular*
1034 *Phylogenetics and Evolution* 124: 1–9. <https://doi.org/10.1016/j.ympev.2018.02.028>
- 1035