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RESEARCH ARTICLE

The phylogenetic position of the bumble bee inquiline *Bombus inexspectatus* and implications for the evolution of social parasitism

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Abstract The high alpine European bumble bee *Bombus* (*Thoracobombus*) *inexspectatus* is one of three taxa in which obligate social parasitism has evolved in bumble bees. Until now, the phylogenetic placement of this species has not been analyzed quantitatively because it is rare in nature. Here a specimen of *B. inexspectatus* is sequenced for five genes to assess its phylogenetic position relative to other bumble bee species. Phylogenetic estimation of *B. inexspectatus* places this species within the subgenus *Thoracobombus*, as expected based on morphology. This provides strong support for the acquisition of social parasitism in *B. inexspectatus* separate from that found in the bumble bees of the subgenus *Psithyrus* and *B.* (*Alpinobombus*) *hyperboreus*. Furthermore, *B. inexspectatus* is not the sister taxon of its host but is a near relative, suggesting a loose but not strict adherence to Emery's rule. *B. inexspectatus* is a sister taxon to a clade including *B. veteranus*, a frequent nest usurper that has been suggested to be a facultative social parasite. The phylogenetic placement of all bumble bee social parasites relative to their hosts is discussed.

Keywords Social parasitism · Inquilinism · *Bombus inexspectatus* · Emery's rule

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Introduction

The bumble bee subgenus *Psithyrus* is a monophyletic clade (Cameron et al., 2007) of obligate social parasites of other bumble bees. All *Psithyrus* invade the nests of other bumble bee species and use resident workers to rear their reproductive offspring. Such parasitism in bumble bees is suspected to have evolved from facultative nest usurpation behavior, in which an invading queen takes over the nest or colony of a resident queen and subsequently rears her own colony. Usurpation is common in bumble bees, occurring in ~10% of nests studied by Richards (1975), and usually occurs among conspecifics or close relatives (Richards, 1975; Sakagami, 1976). Not all bumble bee species are equally likely to usurp nests, but the ones that commonly do are distributed throughout the bumble bee phylogeny, rather than being concentrated in a single clade (Sakagami, 1976; Cameron et al., 2007). This widespread tendency of facultative nest usurpation in bumble bees may have contributed to the independent evolution of obligate workerless social parasitism in two other lineages of bumble bees besides *Psithyrus*: the species *Bombus hyperboreus* and *Bombus inexspectatus*.

Bombus hyberboreus is a social parasite of other members of its subgenus *Alpinobombus*, including *Bombus polaris* (Milliron and Oliver, 1966; Richards, 1973; Pape, 1983) and *Bombus alpinus* (Stenström and Bergmann, 1998), and of the more distantly related *Pyrobombus* (Cameron et al., 2007) including *Bombus jonellus* (Bergwall, 1970) and *Bombus lapponicus glacialis* (Berezin, 1994). *Alpinobombus* occurs in arctic and high alpine regions and therefore must produce small colonies during a brief window of favorable climatic conditions. Such drastically reduced periods of colony production between founding and reproductive stages may have provided the stimulus for *B. hyperboreus* to forego its own worker production and instead produce new gynes and males using the workers in the nests of other species. Although *B. hyperboreus* is recognized primarily as an obligate social parasite, a few workers have been recorded and reproductive females collect pollen, suggesting that *B. hyperboreus* occasionally practices a non-parasitic lifestyle and may contribute to the work of invaded nests (Yarrow, 1970).

The other established case of obligate social parasitism in bumble bees, that of *Bombus* (*Thoracobombus*) *inexspectatus,* is more similar to *Psithyrus*. *B. inexspectatus* is a rare species found at the higher altitudes in the French and Italian Alps (1,450–2,100 m) and in the Cantabrian Mountains of northern Spain (1,181–1,625 m) (Yarrow, 1970). Yarrow (1970) hypothesized that this species is an obligate social parasite, as no true workers were located among 36 female specimens from numerous localities and collecting events. Unlike *B. hyperboreus*, this species appears to have lost morphological traits important for colony founding and maintenance, including loss of wax production and a reduction of the hair-like structures of the hind leg used to gather pollen; no collected bees had pollen on their corbiculae. This species has an expanded region between the eyes and the pronotum, which in *Psithyrus* has been suggested to protect the sensitive area between the head and mesosoma during defensive attacks by hosts (Yarrow, 1970).

Bombus inexspectatus was described relatively recently (Tkalců, 1963), likely because it is rare and difficult to distinguish from close relatives *B. sylvarum* and *B. ruderarius*. *Bombus ruderarius* is sympatric with *B. inexspectatus* throughout its range and was predicted by Yarrow (1970) to be its host. Müller (2006) provided evidence for this hypothesis by finding a newly emerged *B. inexspectatus* female among *B. ruderarius* reproductives and workers in a late stage *B. ruderarius* nest.

Emery (1909) observed in ants that most social parasites are close relatives of their hosts. According to the subsequently assigned Emery's rule (Le Masne, 1956), social parasites are predicted to be either the closest species to their hosts (strict interpretation of Emery's rule) or close relatives (loose interpretation of Emery's rule; Ward, 1989). Primary hypotheses for the evolution of social parasitism according to Emery's rule involve either the evolution of a socially parasitic "cheater" lineage from within its host lineage (hence sympatric speciation), or acquisition of the socially parasitic lifestyle in a descendent species on the sister species or a near relative after allopatric speciation from its sister taxon (Hölldobler and Wilson, 1990). This is promoted by inherited similarities in life history traits and species recognition.

Emery's rule may apply to *B. inexspectatus* given the close morphological similarity between it and its host. The phylogenetic relationships needed to resolve whether *B. inexspectatus* adheres to the strict form of Emery's rule have been uncertain due to the difficulty of obtaining specimens of *B. inexspectatus*. Here, we assess the phylogenetic position of *B. inexspectatus* using sequences from five genes and determine the degree to which *B. inexspectatus* adheres to Emery's rule. Given the near completeness of taxon representation in the phylogeny, a sister relationship of *B inexspectatus* to host *B. ruderarius* would support the strict form of Emery's rule and provide potential evidence for *B. inexspectatus* evolving in sympatry with its host. If *B. inexspectatus* is a close relative but not the sister taxon to its host, we would conclude that a loose form of Emery's rule applies and that sympatric speciation is unlikely. Using the expanded phylogeny of bumble bees, we summarize the relationship of all bumble bee social parasites relative to their hosts and discuss their evolution in the context of Emery's rule.

Materials and methods

To test Emery's rule and expand the species-level phylogeny of bumble bees with the inclusion of *B. inexspectatus*, we sequenced a male *B. inexspectatus* from Valleve (Bergamo), Italy (elev. 1,960 m) for all five genes included in Cameron et al. (2007), including mitochondrial ribosomal 16S, elongation factor-1α, LW rhodopsin, arginine kinase, and phosphoenolpyruvate carboxykinase, amounting to 3,839 total aligned bases (Accession numbers FJ159106–FJ159111). Extraction procedures, primer sets, PCR conditions, and sequencing protocols follow Cameron et al. (2007). To assess its affinity to the Italian specimen, we also sequenced 16S (496 nts) for a specimen of *B. inexspectatus* acquired from Ristolas, France (1,670 m), which was identified by a different taxonomist.

We performed neighbor-joining phylogenetic analyses on these *B. inexspectatus* sequences and the sequences of all bumble bee species collected by Cameron et al. (2007) to determine whether *B. inexspectatus* falls within its assigned subgenus *Thoracobombus*. When this was confirmed, we performed phylogenetic analyses of the *B. inexspectatus* sequences with 28 other species of *Thoracobombus* s.l. (Williams et al., 2008) sequenced by Cameron et al. (2007), with all descendent species of the clade including *B. humilis* and *B. dahlbomii* (Fig. 1). The tree was obtained using Bayesian phylogenetic inference performed in MrBayes v.3.1.2 (Ronquist and Huelsenbeck, 2003) using models inferred with Modeltest (Posada and Crandall, 1998) for each of the following partitions: 16S (model = GTR + I + Γ), EF-1α exons (HKY + I), EF-1α introns (HKY + Γ), opsin exons (HKY), opsin introns (GTR), ArgK exons (GTR + I), ArgK introns (GTR),

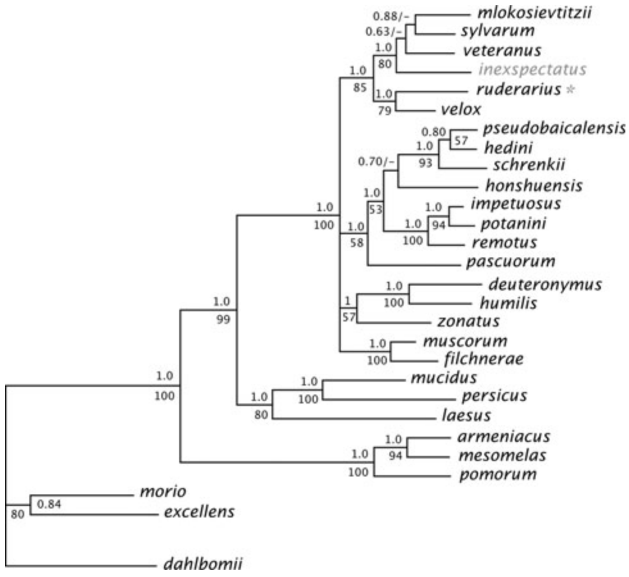

Fig. 1 Bayesian tree based on DNA sequences of five gene fragments, with *B. inexspectatus* highlighted in *gray*. The recorded host for *B. inexspectatus*, *B. ruderarius*, is marked with an *asterisk*. Bayesian posterior probabilities are indicated above and parsimony bootstrap values are indicated *below each branch*, with bootstrap values only shown if >50

PEPCK exons (HKY + I), and PEPCK introns (GTR). We ran three separate Bayesian analyses each with 10,000,000 generations, sampling trees every 500 generations, and using default temperatures and priors. Trees from each of the three analyses converged onto the same log likelihood values within the first 1,000,000 generations, suggested by standard deviations of split frequencies <0.005 and diagnostics in the program Tracer 1.4 (e.g., log likelihood plots, measures of sample independence, and probability densities) (Rambaut and Drummond, 2007). We compiled the resulting trees from the three runs (minus burn-in trees from the first 1,000,000 generations) into a consensus topology. Consensus trees compiled separately from each run had identical cladograms and support values. We obtained additional clade support values based on parsimony criteria using parsimony bootstrapping with 500 pseudoreplicates from a heuristic search with 100 random additions and TBR branch swapping run in the program PAUP* version 4.0 beta 10 (Swofford, 2003).

Results

The phylogeny supports *B. inexspectatus* as a member of the clade that includes *B. sylvarum*, *B. mlokosievitzii*, and *B. veteranus* (posterior probability = 1.0, bootstrap value = 80, Fig. 1). Relationships among the taxa within this clade are not strongly conclusive, however, due to weak support. *B. ruderarius*, the only confirmed host for *B. inexspectatus,* is not the sister taxon to *B. inexspectatus*, but is a near relative; *B. ruderarius* is resolved as a member of the sister clade to the *B. inexspectatus*–*B. sylvarum* clade. The *B. inexspectatus* specimens from France and Italy were identical in 16S sequence, supporting species identification and affinity between the populations.

Discussion

DNA evidence resolves *B. inexspectatus* within the *Thoracobombus* species group, a result consistent with earlier conclusions based on morphology (Williams, 1998). This supports the independent acquisition of obligate social parasitism separate from that of *Psithyrus* and *B. hyperboreus* (Fig. 2). These results are consistent with the loose, but not the strict interpretation of Emery's rule. Because the sister taxa of *B. inexspectatus* are not obligate social parasites, it is most parsimonious to conclude that social parasitism was gained along the *B. inexspectatus* branch. Therefore, social parasitism was acquired after the lineages containing *B. inexspectatus* split from that containing its host *B. ruderarius* (Fig. 1). We thus conclude that sympatric speciation of *B. inexspectatus* from its host is unlikely.

The *Thoracobombus* s.s. species group (Williams, 1998) is particularly prone to usurpation (Voveikov, 1953; Sakagami and Nishijima, 1973). *Bombus sylvarum* and *B. veteranus*, sister taxa to *B. inexspectatus*, as well as the more distant *B. ruderarius*, have all been observed to usurp nests of conspecifics. *B. veteranus* also frequently usurps nests of closely related species, including *B. ruderarius*, *B. sylvarum*, and *B. humilis* (Voveikov, 1953), and was suggested by Voveikov (1953) to be a facultative social parasite. Although *B. veteranus* workers are not infrequent, the species is rare and found in close geographical association with potential hosts *B. sylvarum* and *B. humilis* (P. Rasmont, pers. comm.). The tendency of the species group containing *B. inexspectatus* to usurp nests of near relatives suggests that *B. inexspectatus* likely developed social parasitism from an inherited tendency to usurp nests of related species. Non-obligate socially parasitic tendencies of sister lineages have also been observed in socially parasitic ant lineages (Ward, 1989; Ward, 1996), suggesting that obligate inquilinism can evolve as an extreme of a behavioral cline. *B. inexspectatus* does not overlap much in altitudinal distribution with other members of its clade. *Bombus ruderarius* may have become a host for *B. inexspectatus* because it is the only near relative with similar distribution and niche preferences. Similar to *B. hyperboreus*, the evolution of inquilinism in *B. inexspectatus* may have been facilitated by short seasonality resulting from extreme altitudes. Further observations may reveal *B. inexspectatus* to be parasitic on other relatives besides

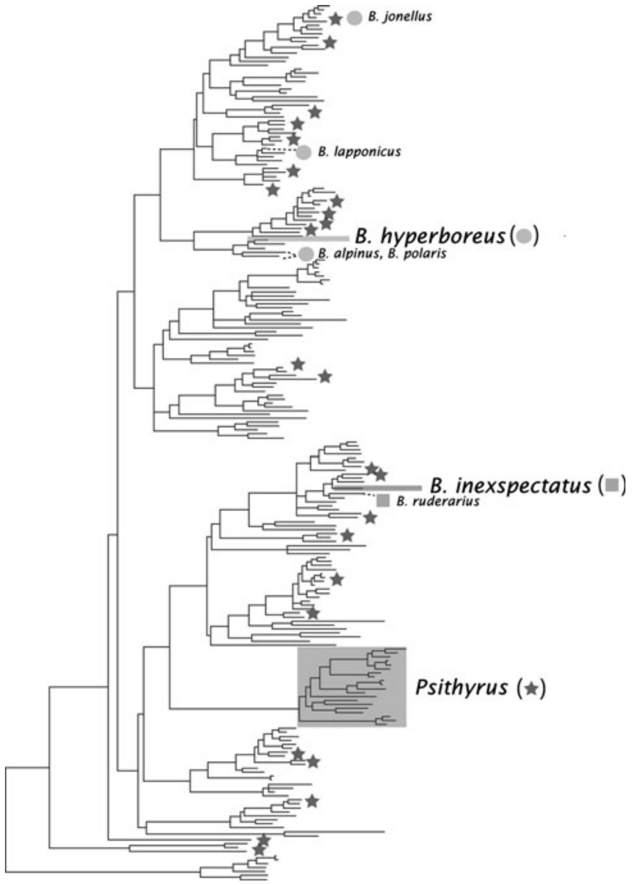

Fig. 2 Position within a nearly comprehensive species-level *Bombus* phylogeny of the three obligately socially parasitic lineages (*shaded rectangles*; *B. hyperboreus*, *B. inexspectatus*, and *Psithyrus*) and their host species, indicated with the respective shapes for each parasite (e.g., *stars* indicate hosts of *Psithyrus*). The position of *B. inexspectatus* was drawn into the phylogeny presented in Cameron et al. (2007). Indicated hosts for *Psithyrus* are those whose nests have been observed to contain reproducing *Psithyrus* (Williams, 2008) and likely only partially represent the true host diversity

B. ruderarius where they co-occur. In *Psithyrus*, parasitism of a single host is rare; they tend to invade nests of inter-related species or not be lineage specific in their nest invasion (Williams, 2008).

These data add to mounting evidence of the variable means by which social parasitism has been acquired in connection with host species. For example, studies in other social insects have revealed that while inquiline social parasites usually parasitize members of the same tribe and genus (cf. Huang and Dornhaus, 2008), the phylogenetic position of parasites relative to their hosts can be quite diverse, including social parasitism of sister species and thus potential sympatric speciation from hosts (e.g., Savolainen and Vepsäläinen, 2003 (*Myrmica* ants); Sumner et al., 2004 (*Acromyrmex* ants); Smith et al., 2007 (allodapine bees) and social parasitism of close relatives but not sister species (e.g., Choudhary et al., 1994 (polistine wasps); Ward, 1996 (*Pseudomyrmex* ants); Sumner et al., 2004 (*Acromyrmex* ants); Smith et al., 2007 (allodapine bees)).

In bumble bees, the obligately socially parasitic subgenus *Psithyrus* split relatively early from its sister lineage, which was inferred by Hines (2008) to have been ~20 million years ago (Ma) relative to the ~34 million year crown age of all extant bumble bees. This split was followed by a much more recent radiation of the ~29 extant *Psithyrus* species beginning ~9 Ma. In contrast to the more recently evolving *B. inexspectatus* and *B. hyperboreus*, this ~11 Ma gap obfuscates events leading to social parasitism in *Psithyrus* because it increases the potential for extinction and host switching. Nonetheless, we can infer that because *Psithyrus* is monophyletic and attacks hosts from across the bumble bee phylogeny with little cophylogenetic pattern (Williams, 2008; Fig. 2), it likely colonized diverse host lineages after evolving social parasitism. *B. hyperboreus*, like *B. inexspectatus*, appears to adhere to a loose form of Emery's rule, as its hosts include sympatric close relatives, but not its closest sister taxa (Fig. 2). The host species of *B. hyperboreus*, however, include the few species that are capable of living in such cold climates, so the phylogenetic proximity of parasite to hosts for this species may be largely a product of the phylogenetic signature of niche preferences. None of the independent acquisitions of social parasitism in bumble bees is inferred to have evolved via the strict interpretation of Emery's rule, and all are unlikely to have arisen via sympatric speciation. Instead it is more likely that bumble bee social parasites evolved as obligate social parasites on congeners with similar niche preferences.

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