

EXPERIMENTAL CONFIRMATION THAT
PTEROMALUS APUM (RETZIUS) (HYM., PTEROMALIDAE)
PARASITIZES BOTH
LEAF-CUTTER BEES (HYM., MEGACHILIDAE) AND
FRITILLARY BUTTERFLIES (LEP., NYMPHALIDAE)

BY M. R. SHAW

INTRODUCTION

Askew & Shaw's (1997) rather surprising conclusion that *Pteromalus apum* (Retzius), a well known gregarious parasitoid in cocoons of megachilid bees, also parasitizes the pupae of certain butterflies was reached on purely morphological grounds following an examination of reared material. Here I report experimental confirmation of that host range.

MATERIALS AND METHODS

Several cocoons of an unidentified species of *Megachile* (Hym., Megachilidae) were found in a rotten *Betula* stump at Bagshot Heath, Surrey (SU8963) on 15.iv.2000 by Matt Smith, who kindly handed them to me because one slightly ruptured cocoon was seen to contain recently formed (still whitish) chalcidoid pupae. This and a second cocoon produced respectively ca 35 rather uniformly sized females of *P. apum* on 25.iv.2000 ('brood 1') and a much larger mixed sex brood (ca 20♀ 73♂) in which size was much more variable on 1.v.2000 ('brood 2').

Both broods were kept intact and each fed in a 7.5 × 2.5cm corked tube on 1 : 3 honey : water from their dates of emergence until 3.v.2000 when about 12 males from brood 2 were transferred to the tube containing brood 1. Much courtship was seen in both tubes resulting in at least a few matings. The tubes containing *P. apum* were kept in a cool detached outdoor wooden shed, under natural light and with feeding *ad libitum*, until females were removed for experiments (in the case of brood 2 only the large females, equivalent in size to those of brood 1, were used). One female from brood 2 was dissected on 3.v.2000, two days after eclosion, and found to contain (? nearly) mature eggs.

Larvae of the butterfly species *Euphydryas aurinia* (Rottemburg), *Melitaea cinxia* (Linnaeus) and *Mellicta athalia* (Rottemburg) (all Nymphalidae: Melitaeinae) were obtained from livestock dealers. All were reputed to be of British origin. Larvae of *Aglais urticae* (Linnaeus) (Nymphalidae: Vanessinae) and *Thymelicus lineola* (Ochsenheimer) (Hesperiidae) were collected from the wild (Norfolk). Larvae were placed singly with foodplant in closed 14 × 8 × 6cm plastic boxes, the floors of which were generously lined with absorbent tissue sufficient to prevent the condensation of water. A small touch of honey was applied near each of the four corners of the lid, and a single female *P. apum* was introduced.

The boxes were then kept indoors at approximately 18–22°C and inspected several times each day. The female parasitoids (and all foodplant and lining) were removed 4 or more days after host pupation, and the host pupae left under dry conditions in the box. (The experimental procedure using *T. lineola* varies from this: see Results.)

RESULTS

1. *Euphydryas aurinia*, on *Lonicera* cv. Larvae in their final instar were received on 3.v.2000, when six replicates were set up, in each case with a female *P. apum* from brood 2. The hosts pupated on 7 (×2), 8 (×2), 9 and 10.v.2000, and oviposition was seen in each case. Broods of 35♂, 46♂, 61♂, 61♂, 62♂ and 65♂ *P. apum* emerged in the period 6–8.vi.2000.
2. *Melिताea cinxia*, on *Plantago lanceolata* L. Larvae in their final instar were received on 5.v.2000, when ten replicates were set up, five with females of *P. apum* from brood 1 and five with females from brood 2. Five of the host larvae proved to have been parasitized by *Cotesia melitaeorum* (Wilkinson) (Hym., Braconidae) and a further 2 larvae harboured a pathogenic fungus and similarly died before reaching the pupal stage. Three larvae pupated 12–18.v.2000 (two with females from brood 1, one with a brood 2 female), and oviposition into all three was seen. One (with a brood 1 female) died, probably from the pathogenic fungus, and broods of 85♂ (brood 1 female) and 81♂ + 1 gynandromorph (mostly ♂ but with predominately ♀ gaster) (brood 2 female) *P. apum* emerged on 13.vi.2000 and 11.vi.2000 respectively.
3. *Mellicta athalia*, on *Plantago lanceolata*. Two larvae were received, in their antepenultimate instars, on 19.v.2000 and immediately set up with females of *P. apum* (one each from broods 1 and 2). Both hosts moulted to their penultimate instars around 22.v.2000 but one then died. The survivor (with female from brood 2) moulted to its final instar on 29.v.2000 and pupated overnight 8/9.vi.2000. Oviposition was not seen, but 16♂ *P. apum* emerged sometime after 10.vii.2000 (unobserved).
4. *Aglais urticae*, on *Urtica dioica*. Two larvae collected on 23.vi.2000 were established with females of *P. apum* (one from each brood) shortly before becoming prepupal on 25.vi.2000. They pupated on 28 and 29.vi.2000; ovipositions were seen into both, and 42♂ (from brood 2 female) and 39♀ 12♂ (from brood 1 female) *P. apum* emerged on 27.vii.2000 and 28.vii.2000 respectively.
5. *Thymelicus lineola*, on *Holcus mollis*. Unsatisfactorily, only prepupae and very fresh pupae were available. One of each, on foodplant, was placed in a 7.5 × 2.5cm corked glass tube (with honey : water) with one female *P. apum* from brood 1 on 7.vii.2000. No interest was noted over 3 days, after which the assemblage (now containing 2 pupae) was left unobserved. Both pupae produced adult butterflies.

PARASITOID BEHAVIOUR

The female parasitoids appeared to be aware of the presence of feeding larvae and occasionally antennated them and/or rested nearby. Usually, however, the parasitoid moved and rested in the box largely independently of the host larva until the latter approached apolysis, when the parasitoid's behaviour changed markedly. The female *P. apum* then almost invariably kept very close to the host—though typically about 1 cm distant rather than on it. Antennation of prepupae was regularly seen, and on a few occasions the host was walked over, but periods of tactile contact were usually brief. (This is in contrast to *Pteromalus puparum* (Linnaeus), which normally stands on the prepupal host, sometimes continuously for a day or more, while it waits for it to moult to the pupal stage). As soon as the host pupates, however, the female parasitoid mounts it and stays in that position while it oviposits through the soft cuticle. No particular part of the host appears to be selected, though intersegmental membranes were possibly sought (not clearly observed). Oviposition is not rapid (bouts last at least several minutes, but none was observed in its entirety), and the pupae were usually visited and oviposited into several times over 2–3 days (whether this would happen in the wild, under less constrained conditions, is unclear), by which time the parasitized pupae had lost their ability to twitch or wriggle. All except one of the broods reared eclosed as adults through a single hole in the host's pupal cuticle—the exception was the smallest of the broods from *E. aurinia* i.e. involving the largest individuals, in which two holes were made.

While almost all the hosts available were in their final instars when experiments were started, the two *M. athalia* larvae exposed from antepenultimate instars onwards revealed that the parasitoid is intensely interested in hosts approaching apolysis even if the moult will not be to the pupal stage. Even at the antepenultimate/penultimate instar moult both *M. athalia* larvae received heavy bouts of antennation and both were climbed onto, in one case the parasitoid holding tight for several minutes while the host repeatedly jerked. Once the moult was completed, however, the parasitoid lost interest until the next apolysis. The *M. cinxia* that were already parasitised by *C. melitaearum* were also quite revealing: when these host larvae ceased feeding and spun the feeble retreats in which they rested until the endoparasitic *Cotesia* larvae egressed from them, they became attractive to the *P. apum* females and remained so for the next few days (the stricken *M. cinxia* larvae did not die until a few days after the *Cotesia* larvae had left them and made their cocoons close by). At the time it appeared possible that the *Cotesia* cocoons were the source of attraction (i.e. that *P. apum* may be capable of acting as a pseudohyperparasitoid) and so the *P. apum* was left with them until darkening contents indicated that adults were about to eclose from the cocoons, but as all cocoons produced *C. melitaearum* adults this was evidently not the case. The lack of any mortality in these cocoons also suggested that they had not been used for host-feeding (which was not observed in any context). It therefore

seems probable that biochemical changes in the parasitized host larva as the *Cotesia* larvae prepared to leave it had a similar effect on *P. apum* to those heralding ecdysis in healthy hosts.

DISCUSSION

These results demonstrate very clearly that *P. apum* individuals that had fed as larvae on *Megachile* are both willing to parasitize certain butterfly pupae and capable of doing so with a high rate of success. The range of Melitaeinae genera (*Euphydryas*, *Melitaea* and *Mellicta*) used experimentally matches that for which wild broods were available to Askew & Shaw (1997) and it appears that—despite considerable morphological variation within *P. apum*—their conclusion that only one taxon is involved is amply corroborated.

The range of other butterfly taxa covered by the experiments reported above was not great, but it appears probable that the hesperiid *Thymelicus lineola* is outside the host range of *P. apum* whereas the nymphalid *Aglais urticae* is within it. *A. urticae* is a much-reared species, though not usually collected as a pupa, but there appears to be no published record of its serving as host for *P. apum*. However, it may be that broods of *P. apum* from some butterfly pupae, perhaps including *A. urticae*, have been erroneously presumed to be invariably only the well-known *P. puparum* (which is superficially rather similar—see Askew & Shaw, 1997). Careful examination of *Pteromalus* specimens reared from butterfly pupae in Britain and W. Europe will no doubt shed light on the host range of *P. apum* in the future. The possibility that *P. apum* tends to alternate between *Megachile* as its overwintering host and butterfly pupae in its summer generation might also be worth examining: the parasitoid is well known to occur in areas where there are no Melitaeinae so, if such an alternation of hosts does take place, this would certainly imply a wider host range that includes common and widespread butterfly hosts, of which *A. urticae* may be one.

It is interesting that *P. apum* attacks butterfly pupae at a precise stage in their development—just after the larval-pupal ecdysis. These pupae are formed in more or less exposed situations. In contrast, *Megachile* species feed in a well-fabricated cell that is at least partly concealed, eventually pupating inside it in a fairly tough cocoon. Even if such hosts are attacked before cocoon construction (though probably they are not), to reach its bee hosts *P. apum* must either enter this structure or oviposit through it. Either way, attacking both *Megachile* and butterfly pupae seems to demand an unusually high diversification of oviposition behaviour.

The sexual composition of the experimentally reared material was disappointing; as morphometric comparisons between broods from different hosts was frustrated by a lack of females—only one mixed sex brood (ex *A. urticae*) resulting. Indeed, the compositions of the two parent broods, from *Megachile*, seemed surprising and it is possible that the stock was experiencing some abnormal genetic effects in sex-determination (as

can happen from inbreeding). The presence of a gynandromorph adds, perhaps, to the suspicion that some of the broods may have included diploid males.

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REFERENCE

Askew, R.R. & Shaw, M.R., 1997, *Pteromalus apum* (Retzius) and other pteromalid (Hym.) primary parasitoids of butterfly pupae in Western Europe, with a key, *Entomologist's mon. Mag.*, 133: 67-72.

National Museums of Scotland, Chambers Street, Edinburgh EH1 1JF.
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Does disease threat cause colony emigrations in the leafcutting ant Atta colombica (Guerin)? — I visited the Smithsonian Tropical Research Institute, Gamboa, Panama during May 2000 to study waste management in leafcutting ants (Hym., Formicidae (Attini), *Acromyrmex*, *Atta*). These Neotropical ants culture a symbiotic fungus within their underground nests, which they use as food for their brood and to a lesser extent for the workers (for details of leafcutting ant biology see Hölldobler & Wilson, 1990, *The Ants*, Harvard; Weber, 1972, *Gardening Ants: the Attines*, American Philosophical Society, Philadelphia, Pennsylvania). Queens can live for 15 years or more and colonies can become very large; a mature *Atta* nest can have a million or more workers (Weber, 1972, as above). As such the nest represents a major investment and colonies generally remain within one nest for their entire life. Colony emigrations have been briefly reported in leafcutters (once in *Acromyrmex histrix* (Latreille) and *Atta cephalotes* (L.)) but in both cases the emigration followed strong physical disturbance (Weber, 1972, as above). Other social insects relocate colonies in response to disturbance and other factors. For example hornets (*Vespa crabro* L.) will relocate if a nest site becomes too small (Matsuura & Yamane, 1984, *Biology of the Vespine wasps*, Berlin) and Asian honeybees (*Apis dorsata* (F.) and *A. florea* (F.)) relocate in response to food shortages (Ruttner, 1988, *Biogeography and taxonomy of honeybees*, Springer-Verlag, Berlin). In *Apis dorsata* this has become a migratory pattern with relocation becoming an integral component of their life history (Ruttner, 1988, *ibid.*). Here I report evidence that disease may stimulate emigrations in leafcutting ants.

I discovered a colony on the morning of 13.v. situated on the slope of a shallow road cutting between Gamboa and Summit, Panama, about 5m from the apron. The nest was visible even from a moving vehicle because the waste heap, located outside the nest in *Atta colombica* (Guerin), was covered with a white cotton-like fungal mycelium, which ramified throughout the heap (dimensions c. 2m x 1.3m x 0.4m deep). This fungus was later identified by A. Dalling (of S.T.R.I.) as *Escovopsis*. *Escovopsis* is a virulent parasitic fungus of the symbiotic fungus and is known to destroy colonies (Currie *et al.*, 1999, The agricultural pathology of ant fungus gardens, *Proc. natn. Acad. Sci. U.S.A.*, 96: 7998-8002). The nest itself was mature and local sources suggested that it was at least 5 years old. There were no signs of physical disturbance. At this stage the surface activity was confined to waste removal, with c. 250 workers per minute leaving the nest carrying dead and dying fungus garden fragments. This is double the waste removal activity I have witnessed in nests of similar age and size (Hart & Rainieks, Waste management in the leafcutting ant *Atta colombica*, *Behavioral Ecology*, in press). Workers of the nest were not foraging. At 4pm a dramatic change had taken place and a file of workers was leaving the nest and heading down a foraging trail into the forest. Workers of all sizes were present and at least

80% of the workers were carrying brood (as eggs, larvae and pupae). I followed the file of workers a total distance of 190m to a site above a stream. There had clearly been much recent excavation at this site and the transiting workers were entering the nest via three holes. Interestingly, foragers were already bringing in leaf fragments cut from a tree 10m from the nest. Garbage removal was continuing at the same level at the old nest site. The exodus of workers continued throughout the night, although I was unable to see the queen. By 10am the following day workers were no longer leaving the old nest site although waste removal was still continuing and did so for at least 4 days (at which point I had to cease observation).

I observed similar behaviour in a small *Atta colombica* colony (ca. 10,000 workers), which was leaving a nest site in a hollow log for an underground site 35m away on Pipeline Road, Gamboa, Panama. I could not find any evidence of *Escovopsis* infection in this case although the waste heap was impossible to locate. The log itself was near to the road and disturbance could not be ruled out as a causal factor in this case. The emigration proceeded in a similar manner to the first nest, with foragers already bringing in leaf fragments to the new site whilst the emigration continued. I searched the fungus gardens and the file of workers and was unable to find the queen. Given that the nest was small and that *Atta* queens are large and distinctive I think it is likely that she had left the nest early in the emigration.

Colony emigration is interesting for a number of reasons. Certainly important (with potential insights into pest management strategies) is the question of what conditions stimulate emigration in the first place. Disturbance appears to be important, but I believe that disease threat is also a key factor. Workers can carry small pieces of fungus garden uninfected (or infected below a critical level) with *Escovopsis* in their infrabuccal chamber or held aloft in their mandibles. This uninfected fungus can be used to seed a new garden. Although emigration is a considerable investment (the old nest must be abandoned, a new nest excavated) the colony is completely dependent on its fungus gardens and emigration may be the only way to escape a serious disease threat. We may also ask what factors are important in selecting a new nest site and how is information on potential sites assimilated and integrated at a colony level? I feel that it would be rewarding to study the organisation of the workforce: e.g., which workers go to make the new nest, what stimulates them to leave and what finally stimulates the colony itself to emigrate? Furthermore, the organisation of the emigration itself is likely to prove interesting – for example, at what point does the queen make her crucial but hazardous break for the new nest? Leafcutting ants are an important model system for studying social insect organisation and symbiosis. Colony emigrations could be a valuable tool to gain insight into how colony organisation and function can be integrated to overcome potentially fatal challenges. — ADAM G. HART, Department of Animal and Plant Sciences, University of Sheffield, Sheffield S10 2TN. E-mail: bop98agh@sheffield.ac.uk : July 20th, 2001.

Priocnemis cordivalvata Haupt. (Hym., Pompilidae) and *Crossocerus distinguendus* (Morawitz) (Hym., Sphecidae) found in Yorkshire. — A female of the spider-hunting wasp, *Priocnemis cordivalvata* Haupt, was taken at Cornelian Bay (TA0685), North Yorkshire on 28.vii.2000 by the author. This is the second record for Watsonian Yorkshire. The first record was at Howell Wood (SE4309), South Yorkshire during 1997 by J.D. Coldwell. Cornelian Bay is a coastal site comprising boulder clay cliffs, subject to frequent slippage, with sandstone headlands.

A male of the sphecid wasp, *Crossocerus distinguendus* (Morawitz) was taken at York Cemetery (SE6150) by the author on 27.vii.2000. This is the first record for Watsonian Yorkshire. The species was first recorded in England near Canterbury, Kent, during 1979 (Packer, 1981, *Entomologist's mon. Mag.*, 117: 97–98). It was recorded at Leicester during 1998 (Archer, 2000, *Entomologist's mon. Mag.*, 136: 54). The species has taken 21 years to reach Yorkshire and this York record indicates that the species is continuing to increase its range. — MICHAEL E. ARCHER, 17 Elmfield Terrace, Malton Road, York YO31 1EH, February 11th, 2001.