

Overwintering behaviour of *Diphyus quadripunctorius* (Müller) (Hymenoptera: Ichneumonidae, Ichneumoninae) in south-east Scotland

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ABSTRACT

Overwintering behaviour of adult females of the ichneumonid *Diphyus quadripunctorius* (Müller) in caves and mines in south east Scotland was monitored over two successive winters. This is a univoltine larva-pupal parasitoid of certain *Noctua* species, parasitizing their larvae in spring. The species was found in rather few of the sites investigated, but in one site in particular it was numerous. There the species was present over the period mid-July until the end of March. It remained semi-active through the winter in all these subterranean sites, some of which were prone to flooding. The ambient temperatures were always well above freezing, which probably allowed the insects to move to safety, although some drowned individuals were found. Wider data drawn from the National Museums of Scotland collection suggest that, in S. Europe as well as in Britain, *D. quadripunctorius* enters the sites in which it will eventually overwinter rather soon after emergence from its host pupae in early to mid-summer.

Keywords: Ichneumoninae, overwintering, caves and mines, Scotland

INTRODUCTION

In temperate regions, getting through the winter is a challenge that parasitoid wasps meet in a variety of ways. Some species do it as an immature larva within a still-living host, others as a cocooned pupa or prepupa or even as a pharate adult, and others again as a mated adult female (the males having died after mating). The last method is practised by females of several groups of Ichneumonidae (Broad, Shaw & Fitton 2018), parasitizing their hosts the following spring. The ovaries do not start to produce yolked eggs until then. Although seen sporadically in several ichneumonid subfamilies the habit is especially widespread in the subfamilies Ichneumoninae and Phygadeuontinae (it occurs also in some Braconidae, but it is practised by far fewer taxa in that family). In general terms a range of overwintering sites has been noted (under dead bark, grass tussocks, dense conifer foliage, thatch, etc.), but little detail is recorded of site preferences for individual species. An exception is the widespread and generally abundant ichneumonine *Diphyus quadripunctorius* (Müller). This is the most frequently encountered ichneumonid overwintering in caves and cellars in Europe (Novák, Thirion & Janžekovič 2010; Vas & Kutasi 2016) and was even voted 'Cave animal of the year' in 2017 by the German Speleological Society (<https://hoehlentier.de/en/four-spotted-cave-ichneumon-wasp/>).

In spring the overwintered female parasitizes certain Noctuidae, ovipositing into the well-grown host larva but the resulting parasitoid larva delays its development

and kills its host as a pupa (this is called larva-pupal parasitism, and by allowing the host to continue with its life for a time the parasitoid is termed a koinobiont; *cf.* Broad, Shaw & Fitton 2018). Like almost all Ichneumoninae, the adult *D. quadripunctorius* eventually emerges from the host pupa. After emerging and mating, the adult female parasitoid needs to feed from flowers *etc.* and especially to find a safe site in which to hide until the following spring.

In the course of surveys of caves, mines, old buildings and culverts in Scotland being undertaken to monitor two overwintering moths (Baird & Cubitt 2019a; 2019b), several female examples of *D. quadripunctorius* were encountered by one of us (KB) in three locations in East Lothian and Berwickshire. In this paper we report on their progress and fate through two winters, 2017/2018 and 2018/2019. Smaller numbers of females of two other ichneumonines, *Diphyus palliatorius* (Gravenhorst) and *Exephanes occupator* (Gravenhorst), were also found in one location.

MATERIALS AND METHODS

Study sites

Gullane, East Lothian: Coastal cave: A cave in low sandstone cliffs with an entrance just above high tide. Depth 15m, width 10m (maximum) and height 3m (maximum). The walls were of smooth sandstone, but with deep cracks, crevices and alcoves in which insects could hide from view. There was no flooding during the study period, nor evidence of past flooding.

Cockburnspath, Berwickshire: Coastal coal mine: A straight passage off a rocky shore into low cliffs (Fig. 1). Smooth sandstone walls, with just a few deep cracks. Height 1–1.5m, width 1.5m. Passage continues for 30m before ending in a shaft (5m in diameter), which extends up to cliff top level. No flooding during the study period, but some tidal debris inside suggests occasional flooding by seawater.

Duns, Berwickshire: Copper mines: Two 1.5m wide passages, about 50m apart, cut into rock above the River Whiteadder. Walls of very uneven roughly hewn rock (greywacke and siltstone), but with a very few deep crevices. Duns1: Entrance passage 10m long to a chamber dropping down to continue at a lower level for 5m (submerged in flood water for much of the survey period) and a side passage 10m long. Average height of passageway 1.5m. Duns2: Single passage *c.* 30m long, average height 1.7m. Flood water rose to 1m depth following prolonged rain in both years (Fig. 2).

Survey

Sites were visited regularly, usually at least once per month, between August and April (except for Duns2, which was only located in January 2018 but visited regularly thereafter). Using a bright (10 LED) torch sparingly, the numbers and approximate positions of *D. quadripunctorius* females were noted. In some cases photographs were taken (Olympus Tough TG-4), and as far as possible temperatures were monitored (Elitech RC-5 Data Logger), though efforts to record temperatures comprehensively were frustrated by the theft of equipment.

Depository

Exemplar specimens of the three species encountered were collected for identification by MRS and subsequent deposition in the collection of the National



Fig. 1. — Entrance to Cockburnspath coal mine, Berwickshire.



Fig. 2. — Entrance to Duns2 copper mine, Berwickshire.

Museums of Scotland (NMS). Other specimens in the NMS collection are mentioned in the discussion.

Identification

It should be possible to identify *Diphyus quadripunctorius* (as *Amblyteles quadripunctorius*) using J.F. Perkins's (1959; 1960) keys, which can be downloaded free of charge from the Royal Entomological Society's website (royensoc.co.uk/out of print handbooks), especially if allowance is made for an error at couplet 7 of the key to females of '*Amblyteles*' (Perkins 1960). This should read: 'Hind tibia ... rather long spines present almost to the apex' [not base]. In addition, it should be noted that the pale spots on tergites II and III shown in figs 715 and 716 in that work can be entirely lacking. Otherwise the colouring is fairly constant, and our Fig. 3 would provide a useful check especially if attention is paid to the colour of legs as well as the body.

RESULTS

Presence and behaviour

Diphyus quadripunctorius was found in the same four sites in both overwintering seasons. In both winters, numbers in Duns2 were substantially greater than in the other sites. Of the other ichneumonids recorded, a very few *Diphyus palliatorius* were found in both Duns sites in both years and a single *Exephanes occupator* was found in Duns1 in spring 2018. Observations are summarised in Table 1.

TABLE 1: SUMMARY OF *D. QUADRIPUNCTORIUS* OBSERVATIONS IN FOUR CAVES AND MINES IN EAST LOTHIAN AND BERWICKSHIRE DURING TWO OVERWINTERING SEASONS, 2017/2018 AND 2018/2019.

Recording in 2018/2019 started at the beginning of the season and visits were more regular than in the previous 2017/2018 season.

	2017/2018			2018/2019			Temp °C 2017/18 season	Other ichneumonids (total seen from both winter seasons)
	Max. count	Date first observed	Date last observed	Max. count	Date first observed	Date last observed		
Copper Mine Duns1	1	30.xi.17 (drowned)	–	6	27.vii.18	18.iv.19	Min/max 5.7/7.5 Av. 6.5 (Jan–Feb)	<i>Diphyus palliatorius</i> (1)
Copper Mine Duns2	11	Site first visited 2.i.18	2.iv.18	49	13.vii.18	18.iv.19	Min/max 6.0/7.8 Av. 6.9 (Jan–Feb)	<i>Diphyus palliatorius</i> (4); <i>Exephanes occupator</i> (1)
Cockburnspath Mine	2	11.viii.17	Specimens collected on 21.viii.17	4	29.vii.18	3.iv.19	Min/max 8.5/9.3 Av. 9.0 (Aug–Mar)	–
Gullane Cave	3	12.ix.17	3.iv.18	1	27.i.19	17.iii.19	Min/max 5.1/10.2 Av. 8.1 (Sept–Apr)	–



Figs 3–6. — Female *Diphys quadripunctorius* (Müller): 3, in Duns2 copper mine, Berwickshire on 14.iii.2019. The body is about 15mm long; 4, two specimens in a cave at Gullane, East Lothian on 12.ix.2017; 5 & 6, aggregations at Duns2, Berwickshire on 12.viii.2019.

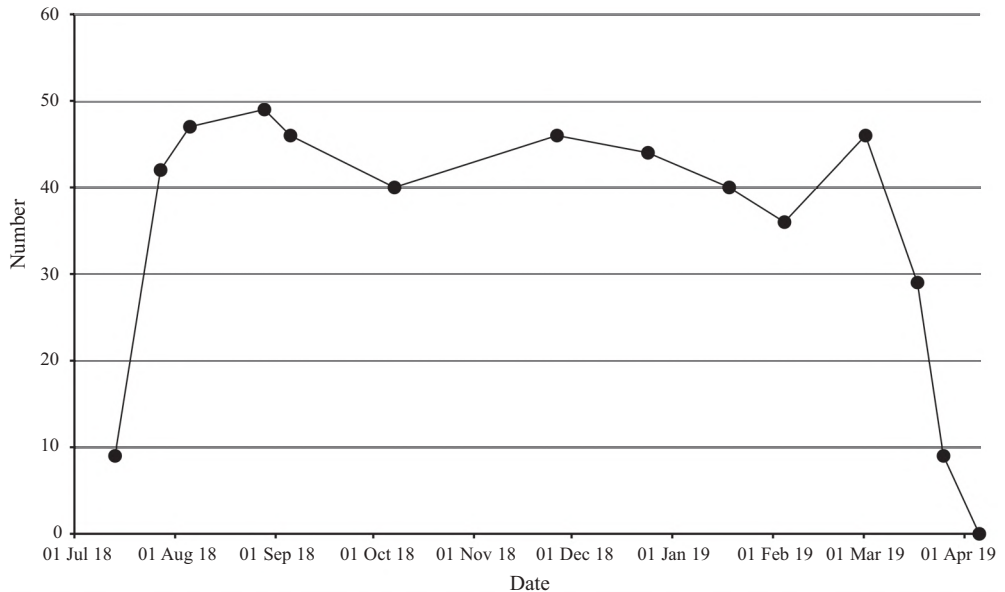


Fig. 7. — Numbers of *D. quadripunctorius* recorded in Duns2 copper mine between July 2018 and April 2019.

The ichneumonids were found between about 8m and 30m from the entrance of the sites, in the same general areas occupied by two species of hibernating moths, Tissue (*Triphosa dubitata* (Linnaeus)) and Herald (*Scoliopteryx libatrix* (Linnaeus)). In the sites where fewer than ten *D. quadripunctorius* were found (Duns1, Gullane and Cockburnspath), almost all individuals were secreted in small cracks or crevices where they remained throughout the winter (Fig. 4). In Duns2 individuals rested in more open areas, but usually where the walls were very uneven, often positioning themselves head-first into the darker corners (Figs 5 & 6).

In the second overwintering season of the study, in Duns2 *D. quadripunctorius* were first recorded on 13.vii.2018. Numbers increased during July and reached a maximum in August. Departure from the mine the following spring began in March and all insects had gone by the beginning of April (Fig. 7). Fluctuation in numbers during the winter was probably due to insects being overlooked on some visits, rather than representing comings and goings.

Over 100 other caves, mines and culverts in southern Scotland were visited during both winter seasons to survey for hibernating moths and 13 of these were visited approximately monthly between September and March. Ichneumonids were not observed in any of them, although all contained Herald moths.

Other relevant British, European and Asian records of D. quadripunctorius

None of the male specimens in National Museums Scotland (NMS) was found in a cave, mine or indoors. All have collection dates in early summer (June to July; often May in S. Europe). In the Natural History Museum, London (NHM) there are two females that were collected in the depth of winter in a cave (Farnham, Surrey, i.1991, *F. Greenaway*). In NMS there is a female collected by David Hodgson from

Scoska Cave, Littondale, North Yorkshire on 26.viii.2006. It was found about 25m into the cave and in a semi-active state. No others have been seen subsequently in Scoska Cave (David G. Hodgson, pers. comm.), although the cave is visited regularly each winter to record the invertebrates within.

There are six reared British specimens in NMS: 3 females and 2 males reared from *Noctua fimbriata* (Schreber) at two sites (*E.C. Pelham-Clinton, M.R. Shaw*) and 1 smaller female reared from the considerably smaller *Noctua interjecta* Hübner (*M. Townsend*). The adults emerged in June and July. The non-reared adults in NMS and other collections mirror the difference in adult size seen in the reared material. (There are no reared specimens in NHM.) Three females in NMS collected by Malaise traps (i.e. active in the field) in Savernake Forest, Wiltshire (*K. Porter*) have the following date ranges: 31.v–13.vi; 22.vi–13.vii; 25.vii–22.viii. Five females in NMS collected in France, Dordogne by Malaise trap (*R.R. Askew*) have dates ranges as follows: 18.v–14.vi (4); 27–31.v (1)

Females in NMS collected entering buildings in Asia and southern Europe have the following data: Turkey, Ankora, Bala, 16.v.1999 (2) (*M.R. Shaw*); France, Dordogne, St Marcel du Perigord, 11.vi (2); 20–23.vi (3) (*R.R. Askew*); Portugal, Algarve, Cerro de Alportel, 1–20.v (8) (*R.M. Lyszkowski*). In addition we have received a report (and photos) of large numbers of females found underground in a former limestone quarry in Indre-et-Loire, France on 7.vii.2018 (*Susan Walter, pers. comm.*).

DISCUSSION

Despite a large number of sites in southern Scotland being surveyed during the two winters for moths, *D. quadripunctorius* was found in only four sites (at three different locations). The patchiness of records, even from sites that are regularly surveyed for invertebrates through the winter, might be explained simply from the relative suitability of the surrounding terrain for the hosts of *D. quadripunctorius*, which seems to use only a limited range of *Noctua* species (perhaps not including the common *Noctua pronuba* (Linnaeus)). This species is regularly parasitized by another larva-pupal ichneumonine, *Amblyteles armatorius* (Forster). However, it seems likely that there will be other significant environmental factors, as yet not fully understood but perhaps including humidity and temperature, which influence site selection.

These insects are easily overlooked, especially when occurring in low numbers and hiding in cracks and crevices, and it is possible that they were present (albeit probably only in small numbers) in a greater number of the examined sites. Indeed, in Gullane, despite regular monitoring of the cave for moths during the 2018/2019 season, it was not until January 2019 that an individual was discovered in a ceiling crevice; presumably overlooked on previous visits. Counts in Duns2 during the course of the winter fluctuated between 36 and 49 individuals; it is very unlikely that there were comings and goings from the mine during this time, but rather that on some visits individuals were overlooked.

The two Duns mines are only about 50m apart in the same woodland habitat, but Duns2 supported notably higher numbers of *D. quadripunctorius* than Duns1 in both winters of the survey. Internally there seems little difference between these passages: both of similar dimensions, cut out of the same rock type, of high humidity and both being partially flooded for much of the winter. Only limited temperature data were recorded from these sites, but Duns1 was slightly cooler than Duns2 during January

and February 2018. This difference was small ($<1^{\circ}\text{C}$) and the temperature ranges through the period overlapped, but it may be the crucial difference. It certainly seems significant that the overwintering sites were considerably less cold than the surrounding countryside. In all sites where it was found, *D. quadripunctorius* tended to occur in small aggregations of 2–8 individuals (Figs 5–6) despite there apparently being sufficient suitable space for them to spread out singly. Aggregating pheromones, common in many insect species, might be released by the early-arriving individuals causing preferential further colonisation of that site over alternative sites close by. However, the small aggregations were still spread through the sites and individuals resting together in the same crevice could be merely a result of optimal conditions in that spot. The possible advantage of aggregation for overwintering female *D. quadripunctorius* is unclear.

In the 2018/2019 season, sites were checked for *Diphyus* from the beginning of July. *Diphyus quadripunctorius* began to arrive at sites in mid-July and there were no further arrivals noted after the beginning of August. This is far in advance of the time needed to escape winter conditions. *Diphyus quadripunctorius* parasitizes the larvae of *Noctua* species, later emerging as adults from their host's pupae. The adult flight time of these moths in Scotland suggests their pupal stage occurs through June and July (Butterfly Conservation East Scotland Branch Website). This is consistent with the reared specimens in NMS, which emerged from their host pupae in June and July, and with British Malaise trap captures. In southern Europe, flight times and also dates at which females are found entering dark spaces such as houses, are earlier. These observations together indicate that female *D. quadripunctorius* spend little time on the wing after emergence, but seek out suitable shelter, at first for aestivation but ultimately in which to overwinter, as soon as they have mated and (presumably) fed.

The *Diphyus* monitored in this study generally remained responsive to external stimuli throughout the overwintering period. Although the torch was used as sparingly as possible, shining light on the insect almost always elicited a response (from slight movement of antennae, to in some cases walking). In Duns2, positions of at least some individuals changed between visits indicating movement also occurred in the absence of potential disturbance from torchlight. The exception was in Gullane in 2017/2018 where one insect spent the winter tucked in a narrow crevice. Here, on three out of four visits in November and December 2017 it was found in the same place with antennae apically curled (characteristic of ichneumonines in deeper winter diapause under colder conditions) and unresponsive to torchlight.

The Duns sites flooded during the winter, the extent and duration of this depending on the amount of precipitation. In both years a few drowned individuals were recovered after a spell of heavy rain or snow-melt (6 in 2017/2018 and 3 in 2018/2019). However, many escaped the rising water levels by relocating higher up the walls, presumably an advantage of remaining partially active throughout the winter. This may be facilitated by the relatively mild temperatures experienced in these caves and mines, and it is even possible that Duns2 was favoured over the slightly colder Duns1 for that reason. The only other overwintering mortality observed was one individual in Duns2 that was found dead in a spider's web in February 2019.

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