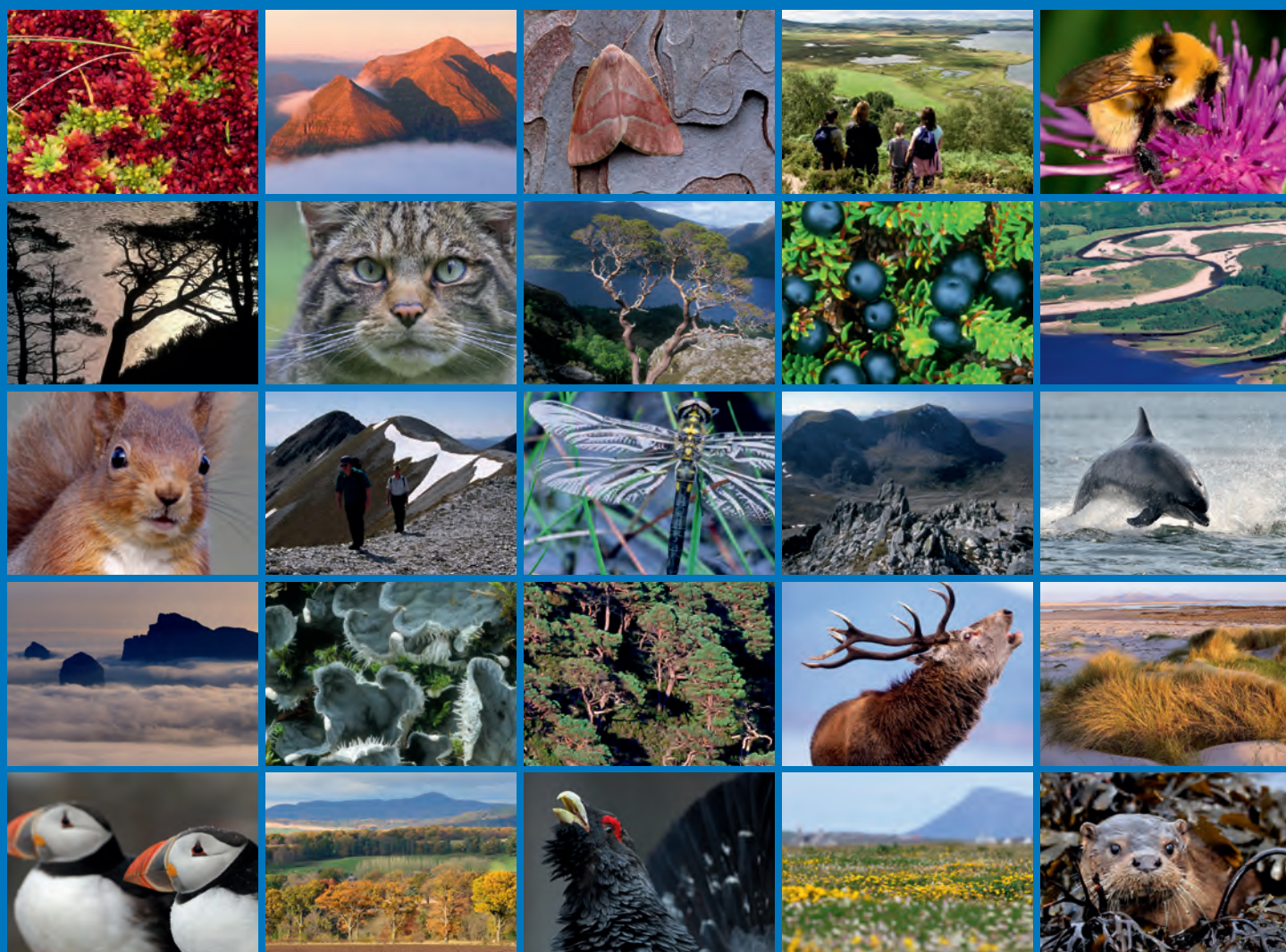


Survey and ecology of *Botanophila fonsecai* Ackland (Diptera, Anthomyiidae), a seed-fly endemic to Scotland





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COMMISSIONED REPORT

Commissioned Report No. 618

**Survey and ecology of *Botanophila fonsecai*
Ackland (Diptera, Anthomyiidae), a seed-fly
endemic to Scotland**

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COMMISSIONED REPORT

Summary

Survey and ecology of *Botanophila fonsecai* Ackland (Diptera, Anthomyiidae), a seed fly endemic to Scotland

Commissioned Report No.: 618

Contractor: D. Gibbs

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Background

The global distribution of the BAP-listed Fonseca's seed fly (*Botanophila fonsecai*) is believed to be restricted to a strip of sand and sparse herbage about 100-m long and a few metres wide on the north shore of the Dornoch Firth. As a putative endemic, *B. fonsecai* should be one of Scotland's biodiversity priorities, in accordance with the Rio Biodiversity Convention. Because of its limited distribution, *B. fonsecai* population is intrinsically small, therefore subject to random demographic fluctuations and environmental vicissitudes. Consequently, *B. fonsecai* is particularly susceptible to extinction. Moreover, its habitat is subject to trampling caused by recreational activities as well as to natural geo-morphological degradation typical of soft, coastal habitats. The protection of this anthomyid fly requires knowledge about its life history so that requirements can be translated into management advice.

Main findings

- All major dunes where permission was obtained from Dornoch Point north to Ferry Links were surveyed from 14 to 17 June 2010. *Botanophila fonsecai* was found at four sites: Dornoch Point, Dornoch Sands (type locality), Dornoch north dunes and Embo dunes.
- Compared to collection data from the 1970s and 1980s, populations were low, which could be interpreted as decline, missed peak of activity or normal population variability.
- No host plants could be ascertained. However, four specimens identified as soon as they were caught seemed to be associated with bare sand amongst lyme grass at the accreting foredune.
- Females were nearly twice as prevalent as males, except at the accreting dune front where males slightly predominated, suggesting these areas are important for courtship or mating.
- Larvae likely to be Anthomyiidae were found in great abundance in the capitula of ragwort and in small numbers in sow-thistle.

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I would also like to thank Dr Athayde Tonhasca for instigating and supporting this project. Also David Patterson, Area Officer, Sutherland and Nathan McLaughlan, Area Officer, Golspie for their help obtaining permission to work on the dunes of East Sutherland.

1 INTRODUCTION

The Fonseca's seed fly (*Botanophila fonsecai*) is a species believed to be restricted to a strip of sand and sparse herbage about 100-m long and a few metres wide on the north shore of the Dornoch Firth. Because of its limited distribution, *B. fonsecai* population is intrinsically small, therefore subject to random demographic fluctuations and environmental vicissitudes. Consequently, *B. fonsecai* is particularly susceptible to extinction. Moreover, its habitat is subject to trampling caused by recreational activities as well as to natural geo-morphological degradation typical of soft, coastal habitats. As a possible endemic species, *B. fonsecai* is one of Scotland's international priorities for conservation, according to the Rio Biodiversity Convention. The protection of this anthomyid fly requires knowledge about its life history so that requirements can be translated into management advice.

2 FIELDWORK

The field work was divided into two parts: three full days in mid-June, to coincide with adult activity, and two whole days in early September when, it was judged, offered the best chance to find full-fed larvae.

2.1 The June Survey

Prior to starting field work, permission was gained to survey as many potentially suitable areas as possible. These included the whole of the extensive dune system of Dornoch Point, the Dunes at Dornoch (Figure 1) and those between Dornoch and Embo. At Embo, only a small area was accessible; the greater part of Coul Links was out of bounds because of landowner objection. At North of Loch Fleet, permission was obtained for all the dunes from Little Ferry to the cart track at the southern end of Golspie golf course (Figure 2).



Figure 1. Dornoch Sands looking south; type locality of B. fonsecai.

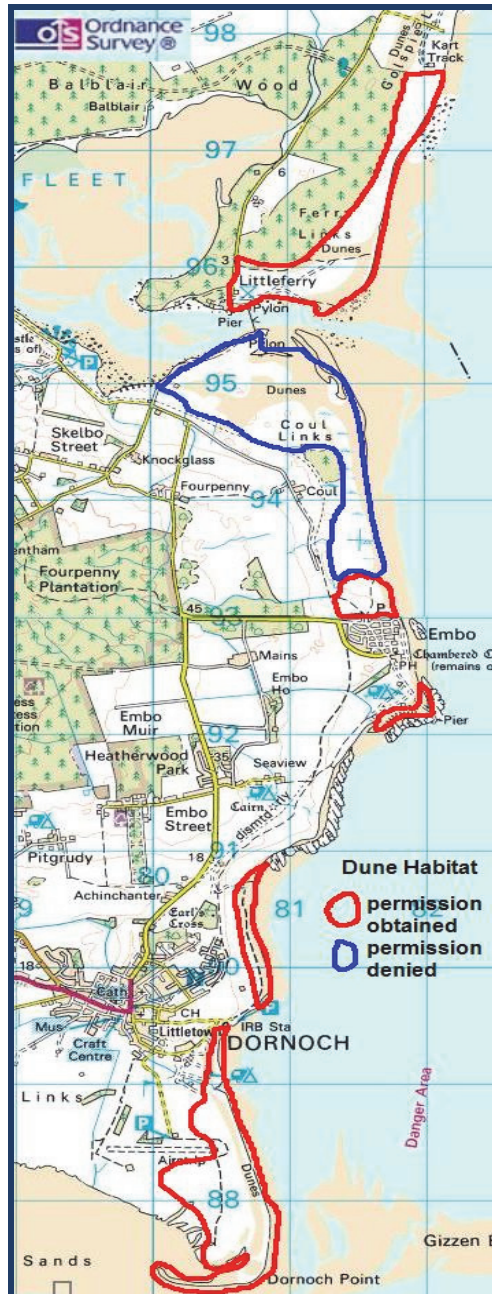


Figure 2. Survey areas.

2.1.1 Field methodology

Because *B. fonsecai* is a small, nondescript anthomyid fly very similar to numerous other small calypterates, it was known in advance that field observations would be very difficult, if not impossible. Indeed this proved to be the case so it was essential to capture flies and examine them in detail. This meant that very few captures could be pinpointed to a precise location; flies could only be identified later by examining the net catch or under the microscope. In order to localise captures as precisely as possible, each of the larger survey areas was divided into zones, usually linear strips parallel to the coast. This was done because, as a general approximation, dune habitat changes from accreting dune on the beach-front to mature dune furthest inland, and *B. fonsecai* may be a specialist of one of these habitat zones.

A sweep-net was used to sample the dune vegetation or to sweep low over bare sand. All potential anthomyid flies were potted and killed with ethyl acetate once the sample zone was completed. With time, it became possible to occasionally identify male *B. fonsecai* in the net. This allowed refining the captures, whereby a small area within a zone was swept, male *B. fonsecai* identified and GPS co-ordinates taken to more precisely locate the catch (all these specimens were kept to ensure accurate identification). Particular attention was paid to flowers, especially yellow composites; small calypterate flies captured from them were retained separately. Additionally, some time was spent searching for leaf-mines in various specialist dune plants and sieving sand in search of puparia.

2.1.2 Lab work

Because of the difficulty in identifying this species in the field, each daily sample was examined under the microscope, and the resulting data were used to guide the search strategy for the next day. Morphological features that could be used in the field were identified for male *B. fonsecai*. For females however, none of the diagnostic features visible at magnification can be seen with a hand lens, while features that can be seen in the field are shared with similar species.

2.2 The September Survey

Because it was unlikely that data from adult flies would help determine their food plant, it was important to find the larvae. There are no reports of *B. fonsecai* rearing or any other clues pointing to a particular plant. All decisions had to be based on inferences from what is known from similar species. In consultation with Mike Ackland, it was deduced that most eggs would be laid between mid-June to mid-July. Assuming that their life-cycle is similar to other closely related *Botanophila*, they would probably be fully formed by mid-August to early October. Thus an early September visit was considered to be the most likely time to find full-fed larvae of *B. fonsecai*.

2.2.1 Field methodology

Effort was concentrated in those areas with greatest abundance of *B. fonsecai* in June. The larvae of *Botanophila* spp. develop in a wide range of situations: dung, rotting plant material, bird nests, fungi, leaf mines, stem mines, root mines and flower heads. *Botanophila fonsecai* is part of the *B. trigemina* Hennig group, which includes *B. setosa* Séguy, *B. trigeminata* Hennig and *B. trigeminoides* Hennig (Ackland, 1989). Unfortunately the host plant is not known for any of these species, making it very difficult to decide which plant species to search. According to Dr Verner Michelsen (guest researcher at the Natural History Museum of Denmark, Anthomyiidae specialist), "my best guess would be that the larva attacks flower heads of a species of Compositae" (pers. comm.). Based on this expert opinion, most September field work was dedicated to searching flower-heads of composites. Larvae were searched in other potential situations as well, especially as leaf and stem miners in a wide range of plants, including non-composite species. A limited amount of time was spent sieving sand around potential host-plants for puparia in case *B. fonsecai* pupariates quickly.

3 RESULTS

3.1 June survey

Survey started on 14 June, when about 1 h was spent with sweeping the type locality in cool but dry conditions. The next three days were spent by surveying the dunes between Dornoch point and Little Ferry, usually in cool, windy conditions, but with some sunshine. Survey work was occasionally made difficult by heavy dew, which remained well into the morning, and an occasional light shower. Overall the conditions were not ideal, and were likely to impact the results to a limited extent. However, it is unlikely that *B. fonsecai* was missed at any site. The results of the survey are summarised in Table 1.

Table 1. Summary of survey results.

Site	Sampling zone	Grid ref	Flies collected (m = males, f = females)	Date	Sample code
Dornoch Sands	D	NH8089	1 m	15/6	
Dornoch north dunes	dune S	NH8090	3 f	16/6	
Dornoch north dunes	dune N	NH8090	1 m	16/6	
Dornoch north dunes	dune S	NH8090	1 f	16/6	
Dornoch Point	G	NH8088	3 m	16/6	
Dornoch Point	H	NH8088	2 m, 3 f	16/6	
Dornoch Point	K	NH8088	1 f	16/6	
Dornoch Point	G	NH8088	5 f	17/6	
Dornoch Point	G	NH80751	1 m	17/6	#363
Dornoch Point	G	NH80794	1 m	17/6	#362
Dornoch Point	G	NH80772	1 m	17/6	#360
Dornoch Point	G	NH80777	1 m	17/6	#361
Dornoch Point	G	NH8088	1 f	17/6	
Dornoch Point	H	NH8088	1 m, 3 f	17/6	
Embo	N of village	NH8193	4 f	17/6	

3.1.1 The type locality, Dornoch Sands

This relatively small area, where all previous specimens of *B. fonsecai* have been taken, is sandwiched between a caravan site and the beach. This tiny patch of habitat measures approximately 700 m north to south, 200 m at its widest point in the southern half and only 80-m wide at the northern end. For the purposes of sampling, it was divided into four parallel zones running from north to south (Figure 3).

Previous observers had found *B. fonsecai* in abundance here, so the first full day of survey was spent thoroughly sweeping the dune grassland and carefully observing small calypterate flies. The zones were chosen to reflect differences in the floristic structure and component species. Zone A, the dune front, was by far the most distinctive area, largely bare sand dominated by lyme grass (*Leymus arenarius*) with scattered *Elymus*, *Atriplex*, *Chenopodium*, *Honkenya* and just occasional groundsel (*Senecio* spp.). Behind this, zone B was very much dominated by marram (*Ammophila arenaria*), and bare sand was much less in evidence except along paths where moss became a major component of the flora. A much greater diversity of plants that have the potential to host *B. fonsecai* were growing

here, notably mouse-ear (*Cerastium* spp.), ragwort (*Senecio* spp.), groundsel, dandelion (*Taraxacum* spp.), cat's-ear (*Hypochaeris radicata*), yellow rattle (*Rhinanthus* spp.), plantain (*Plantago* spp.) and kidney vetch (*Anthyllis vulneraria*). Zone C is stabilised dune where marram is being replaced by other grasses and there are strong populations of ragwort, cat's-ear, groundsel, hawk's-beard (*Crepis* sp.), rosebay willowherb (*Chamerion angustifolium*), mouse-ear, mouse-ear-hawkweed (*Pilosella officinarum*), kidney vetch, white clover (*Trifolium repens*) and plantain. Zone D, furthest from the sea, is very similar to zone C but with more coarse herbs and encroaching woody plants and the moss *Rhytidiadelphus* becoming very conspicuous. Potential host species recorded were mouse-ear, buttercups (*Ranunculus* spp.), vetch (*Vicia* spp.), lesser meadow-rue (*Thalictrum minus*), dandelions, white clover, mouse-ear-hawkweed, hawkweed (*Hieracium* sp.) orchids (*Orchis* spp.), yellow rattle, rosebay willowherb, gorse (*Ulex* spp.), speedwell (*Veronica* spp.), cow parsley (*Anthriscus sylvestris*) and gentians (*Gentianella* spp.).

Only a single male was discovered, in zone D. This is in stark contrast to the situation in the 1960s to 1980s, when *B. fonsecai* were found in greater numbers, and mainly at the dune front.



Figure 3. Dornoch Sands sampling zones.

3.1.2 Dornoch Point

This large area (1.64 km north to south, up to 190 m in breadth) to the south of the type locality was searched over 16-17 June. This area probably has not been searched previously for *B. fonsecai*, certainly not in the 1980s (M. Ackland, pers. comm.). The flora is very much like that found at the type locality but also containing other species, notably sow-thistle (*Sonchus* spp.). This area is much greater than the type locality and it is under much less human disturbance. As before the area was subdivided into zones (Figure 4).



Figure 4. Dornoch Point sampling zones.

This proved to be by far the most favourable area for *B. fonsecai*, where the majority of specimens were taken (Figure 5).



Figure 5. Results of survey at Dornoch Point.

The largest number (13) was found on the accreting dune front (zone G). Here four males were identified in the field, so their point of capture was located fairly precisely. Nine specimens were caught in the hind-dune (zone H), but none on the more northern part of the hind-dune.

All four zones have large areas of bare sand and are dominated by marram and lyme grass with varying amounts of cord grass (*Atriplex* sp.) and *Chenopodium* sp. Capture locations #361 and #363 have sea sandwort while #362 has small amounts of groundsel. The almost complete absence of the most likely host plants, composites, is notable.

None of the male flies captured in zone H were noticed in the field. Although this was partly because they were a little less abundant here, other small calypterates were more diverse and abundant in this richer habitat, making it almost impossible to pick out the nondescript *B. fonsecai*. The flora in zone H is very similar to that in the stabilised dune of Dornoch Sands

just to the north and almost contiguous. Much the same range of potential host plants was noted with plenty of ragwort, cats-ear and sow-thistle.

3.1.3 Dunes north of Dornoch

This is a long narrow strip of dune (1.15 km by about 100 m at most) between the beach and the Royal Dornoch golf course north of the type locality. It is not contiguous with Dornoch Sands with about 370 m of unsuitable habitat between the northern end of Dornoch Sands and the southern extremity of the north dunes. The area was thoroughly surveyed on 16 June 2010, and the samples were localised to three compartments: foredune south, foredune north and hind dune (Figure 6).



Figure 6. Dornoch north dunes sample zones.

While not as productive as Dornoch Point, this area clearly holds a fairly good population of *B. fonsecai*. All specimens were taken on the foredune where there was plenty of bare sand, and a relatively impoverished flora of lyme grass, cord grass, sow-thistle, ragwort and limited amounts of cats-ear and hawkweed. The southern foredune is relatively steep and appears to be suffering from erosion. Contrastingly, the northern foredune has the appearance of a raised beach as if it has recently accreted after profound erosion.

3.1.4 Dunes north of Embo

Although this is a small area (265 x 200 m), it is contiguous with the extensive area of dunes on Coul Links. Unfortunately, permission to survey Coul Links was not forthcoming. About 2 h were spent sampling the whole area. Because the dune front is very steep, there was no

accreting dune to sample separately. All samples were taken on the stabilised hind dune (Figure 7) where there were good populations of ragwort and devil's-bit scabious (*Succisa pratensis*). Also present were marram, sow-thistle, yarrow (*Achillea millefolium*), thistles (*Cirsium* spp.), twayblade (*Listera ovata*), marsh orchids, hawkweed, daisy (*Bellis perennis*), speedwell (*Veronica* sp.), meadowsweet (*Filipendula ulmaria*), mouse-ear hawkweed and a few cats-ear.



Figure 7. Embo survey area.

Four females were found here, which is rather good for so limited an area sampled for just a couple of hours. Also of considerable interest was the apparent absence of *Botanophila seneciella* (Meade), which was present in most other samples. This anthomyid is frequent on dunes around Britain and known to develop in the capitula of ragwort. If *B. fonsecai* is also a ragwort specialist, then there may be some competition between these species.

3.1.5 Ferry Links

The very extensive dune area to the north of Loch Fleet is approximately 2.2 km north to south and 500 m east to west at its widest point. It was searched for several hours on 17 June in rather cool but sunny conditions. Initially the vegetation was wet, making sweeping difficult. These dunes are rather more stabilised than those searched further south, the greater part perhaps best characterised as dune heath with much *Cladonia* lichen and even some heather further inland. The lyme grass and marram-dominated areas here are relatively narrow strips behind the shore-line, and sampling was concentrated in these zones. Most of the potential host plants were present although cats-ear and hawk's-beard (*Crepis* sp.) were not noted.

No *B. fonsecai* were found and indeed the range of flies was rather different from other sample areas. Relatively few anthomyids were found, but they included *B. sericea* and *Delia frontella*, the latter not found elsewhere during this survey.

3.2 September survey

The second visit to Dornoch (31 August to 2 September) allowed two full days of field work on 1 and 2 September. Conditions were very good, warm and dry. The purpose of this visit was to search for *B. fonsecai* larvae and puparia. The four specimens that were fairly narrowly localised during the June survey seemed to be associated with bare sand rather than any potential host plant. Therefore the search had to be wide ranging, no herbaceous plants could be confidently excluded although most time was dedicated to examining composites. To maximise the chance of success, all searches were confined to those areas where most adults had been recorded in June. On 1 September, the whole day was spent on searching the northern half of Dornoch Point while 2 September was split between Dornoch Sands, Dornoch north dunes and Embo dunes.

Across the three areas, especially on Dornoch Point zone G, where *B. fonsecai* captures were most closely spotted in June, some time was spent on sieving sand in search of puparia. These searches were concentrated around grass and herb species and did not reveal any live puparia, even from around *Atriplex*, *Chenopodium* and *Honckenya* which had already been shown to harbour leaf-mining anthomyids. It became evident that this was a most unproductive technique at this time of year, anthomyid species either having already emerged (*Delia* and *Pegomya*) or not yet exited their larval site (probably all *Botanophila*).

By far the greater amount of time was spent opening the seed capsules, flower heads and stems of herbaceous plants in search of larvae. Leaf mines were also searched, but nothing not already found in June (and shown not to be *B. fonsecai*) was discovered. Fungi and other potential sites such as dung were very few, but several fungal fruiting bodies were examined. Only Mycetophilidae larvae were found. The one potential larval development site not thoroughly investigated was the rootstocks of plants. This would require uprooting and destruction of plants, which was considered undesirable before all other potential larval sites have been ruled out.

Phytophagous diptera larvae were found in three plant species: knapweed (*Centaurea nigra*), perennial sow-thistle (*Sonchus arvensis*) and common ragwort (*Senecio vulgaris*).

3.2.1 Knapweed

Larvae and vacated Tephritidae puparia - most likely *Urophora jaceana* (Hering) - were found in a few plants. As it could be confidently concluded that these were not anthomyid larvae, no specimens were retained.

3.2.2 Perennial sow-thistle

This tall, conspicuous composite was present as single plants or large colonies across the survey area, most frequent in zone F of Dornoch Point and the north foredune of the dunes north of Dornoch, but there were large expanses where no plants were found. All isolated plants and a good proportion of large colonies were checked for fly larvae. Fly larvae with the characteristics of Anthomyiidae were found in an isolated plant near the tip of Dornoch Point, in a few of the large number of plants in zone F of Dornoch Point and in several plants along the foredune of the dune north of Dornoch. None were found in the few plants at Embo. Samples were retained from all areas in the hope that the species could be obtained by rearing.

The larvae found in *Sonchus* are almost certainly Anthomyiidae and quite likely *Botanophila*, although they appear to be too large to be *B. fonsecai*. These larvae are most likely to be *Botanophila sonchi* (Hardy), a species frequently found on dunes around Britain.

3.2.3 Common ragwort

Ragwort, thought to be the most probable host plant, was heavily infested with Anthomyiidae larvae, and it soon became easy to spot plants containing well-grown larvae from the frass extruded from between the florets of the flower head (Figure 8).



Figure 8. Ragwort flower heads infested with Anthomyiidae larvae

Infested flowers are usually less symmetrical and the dead florets become stuck together with blackish frass, the pappus never developing. By the time the larvae are fully fed, the capitulum is often swollen and there are few or no dead florets. None of these flower heads contained puparia, but many that had clearly been infected were empty. From this observation it is clear that these anthomyid flies leave their larval development sites once fully-fed to pupariate elsewhere.

These larvae invariably occurred singly with their head orientated downward, and were approximately the size that would be expected for *B. fonsecai* (Figure 9).



Figure 9. Bisected ragwort flower heads, normal (left) and infested (right)

Unfortunately *B. seneciella* (Meade), a species known to develop in the capitula of ragwort, is present at most (probably all) of the survey locations. This fly is very similar in size to *B. fonsecai* so with our current knowledge of Anthomyiide larval morphology, it is impossible to rule this species out. It is also possible that both species are using the same larval development site. A very large number of samples were retained in the hope that some could survive the winter in captivity and emerge the following May/June so permitting identification.

3.3 Results of *ex situ* cultivation

Following cultivation until June 2010, large numbers of *B. seneciella* and a few *B. jacobaeae* emerged from ragwort, and quite a lot of *B. sonchi* from *Sonchus*. Unfortunately no *B. fonsecai* emerged but this exercise has at least shown that the technique used has the potential to be successful.

4 CONCLUSIONS

4.1 Distribution

The first objective of the survey, to confirm the continued survival of *B. fonsecai* at the type locality at Dornoch Sands, was successful, with a single male being discovered here. The assumption that *B. fonsecai* occurs more widely on similar habitat within the area was also confirmed. This survey has extended the distribution of *B. fonsecai* from the 700 m of dunes at the type locality to four localities along 6.3 km of coastline from just north of Embo to the tip of Dornoch Point. If we make the very reasonable assumption that *B. fonsecai* occurs on Coul Links up to Loch Fleet, then *B. fonsecai* would occur along 8.1 km of coastline where suitable habitat exists.

4.2 Status

The status of *B. fonsecai* from the evidence of this survey is less encouraging. The type locality, the only site where *B. fonsecai* had been found or even looked for previously, proved unproductive. Both Fonseca and Ackland found *B. fonsecai* in abundance in the 1970s and 1980s respectively. The single male found at the type locality during this survey suggests a dramatic reduction in population which, if true, would suggest a significant decline in the suitability of the habitat for *B. fonsecai*. However, an alternative possibility is that this survey did not coincide with peak activity of *B. fonsecai*. Although the timing of the survey was chosen to approximately coincide with *B. fonsecai*'s peak flight period as far as could be judged from previous records, this would be far from infallible. The data from which peak flight period was deduced was just two years and it is quite possible that these were not typical years. Even more likely was that 2010 was an atypical season with *B. fonsecai* having already completed much of its flight period by the time this survey started. There is some evidence that this is the case from the preponderance of females found. In many species males emerge first, or at least are in greatest abundance in the early part of the flight period. Females not only tend to emerge a little later, or at least peak a little later, but survive longer after mating while they mature and lay eggs. In 1971 Fonseca found 40 males and 35 females between 15 and 18 June while this survey found 12 males and 21 females between 15 and 17 June. Thus between 1971 and 2010, on the same dates in June, the ratio of males to females is reversed, suggesting that the peak of emergence in 2010 was a week or two before the peak emergence in 1971.

At the other three survey locations where *B. fonsecai* was found, populations seemed to be better than at the type locality. However, even at Dornoch Point, they were considerably less abundant than at the type locality in 1971 and 1984. Even if it is assumed that the 2010 survey missed peak activity of *B. fonsecai*, the occurrence of reasonable numbers on Dornoch Point but not at the contiguous type locality strongly suggests that the favourable habitat of Dornoch Sands has declined. While far from conclusive, these data seem to indicate a decline in the population of *B. fonsecai*, at least in part of its very restricted range.

4.3 Natural history of *B. fonsecai*

This was by far the most tentative part of the project as determining the larval development site in the time available was a very optimistic objective.

Attempts to associate potential host plants with *B. fonsecai* while doing the survey work in June were not successful because it proved impossible to identify *B. fonsecai* in the field. The only specimens that could be pinpointed to any useful extent were the four males found in zone G of Dornoch Point. These limited data suggest that *B. fonsecai* has a requirement for bare sand on accreting foredune. However, the presence of *B. fonsecai* across the stabilised dunes, and the lack of the most probable host plants on the foredune, strongly suggests that *B. fonsecai* does not complete its entire life cycle there. It may be speculated

that males and females gather on the warm, bare sand of the foredune where mating takes place, then mated females disperse across the dunes to mature and lay eggs. Tentative support for this can be seen in those zones where sufficient specimens were found. Foredune zone G yielded seven males and six females, while stabilised dune zone H yielded three males and six females. So zone H is in line with the overall ratio of males to females whereas zone G has more males than females.

At this stage, no conclusion can be reached regarding the host plant. All that can be said is that, if *B. fonsecai* develops in the capitula of a composite species, then this is likely to be ragwort.

5 FURTHER SURVEY

This survey should be seen as a provisional investigation of the status, distribution and ecology of *B. fonsecai*, especially with regard to the last of these objectives.

5.1 Distribution

This was by far the easiest objective to achieve and, within the limited time, was completed as far as was possible. However, there is still much that could be done to discover this species elsewhere around Scotland, or, perhaps more significantly, confirm that it really is endemic to this short length of coastline. There are many potentially suitable expanses of dunes on the east and north coast of Scotland that could harbour this species and would be well worth searching. The following suggestions are in order of priority:

- Coul Links
- Morrich More
- dunes north and south of Brora
- Culbin Dunes, Whiteness Head and Findhorn dunes
- Keiss Links and north coast sites west to Durness
- dunes from Lossiemouth to Aberdeen
- dunes Aberdeen southwards

5.2 Status

Assessing the status or the population viability of *B. fonsecai* will not be an easy task because it requires in the first instance a fairly detailed understanding of its phenology. Dornoch Point would be the best study site from current knowledge as it has the strongest population found during this survey. By using a standardised method, such as timed sweeping, samples could be taken every few days (at least once a week) from the beginning of May to end of July. Water trapping would be a better sampling technique, but because all sites so far found have public access, this method is prone to disturbance. Once these data are plotted, spot surveys of other sites can be compared and an assessment of the relative population deduced. Ideally such a calibration survey would be needed each year the population was assessed, but even without this it should be possible to deduce the state of the population to a reasonable approximation, notwithstanding the potential variability in peak adult emergence.

At the very least the population of *B. fonsecai* at Dornoch Point should be assessed by the use of a standard method about every 5-6 years. Part of such a survey could include floristic and geomorphological assessments of the habitat. Fixed-point photography may be a good way to achieve this.

5.3 Further ecological investigation

Univoltine species are notoriously difficult to rear because of the necessity to bring them through the winter. This is likely to be particularly the case with regard to specialist species that naturally overwinter in habitats a long way from the location where they are being overwintered in captivity.

As the samples taken failed to produce any *B. fonsecai*, attempts to discover the host plant also failed and it is not possible to rule out sow-thistle or ragwort as potential hosts. Most of the larvae collected emerged but none were *B. fonsecai*, so this is good evidence that sow-thistle and ragwort capitula are not the larval development site. However such negative evidence can never be definitive. As the efforts of the 2010 survey did not elucidate the host plant of *B. fonsecai* then two approaches are recommended.

5.3.1 Further host plant field work

A better sampling protocol will have to be developed. Until investigated more thoroughly, the assumption that ragwort or another composite is the host species should be pursued. Rather than taking flower heads of infested plants away for rearing, the potential host plants should be netted *in situ*, including roots several inches deep. Because of the open nature of the sites, these are unlikely to survive undisturbed. For this reason it will probably be necessary to transfer potential host plants to pots to a safe enclosure as close to the source dunes as is possible. These samples will need checking regularly from the beginning of May to end July, and flies removed. If this is not possible, then the netted plants will have to include catching bottles so that emerging flies can be preserved. Similar netting emergence traps can be used for other potential larval development sites such as fungi, strandline debris and dung.

5.3.2 DNA

One way to short-cut the necessity to overwinter fly larvae is to research their DNA. It is likely that results from other families will work well for Anthomyiidae and the same mtDNA sequences could be used to determine co-specificity. However, it would almost certainly be necessary to sequence a range of anthomyids, especially *Botanophila* species likely to be sympatric with *B. fonsecai*. Once it is ascertained that *B. fonsecai* can be conclusively identified from a molecular sequence, then it will be a straightforward matter to associate any larvae that can be found.

6 REFERENCES

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