Imperial College
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Foraging ecology of *Formica rufibarbis* Fab. (Hymenoptera: Formicidae) on Chobham Common and possible implications for surveying this endangered species

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Abstract

We are now approaching what scientists have named "The Sixth Extinction". Ants are considered incredibly important taxa to ecosystems. The red barbed ant (*Formica rufibarbis*) is arguably the rarest animal in Britain and is likely to be the next ant species to disappear from British shores. In the early 20th Century nest sites within England were evident in large areas of heathland in South-east England. Presently only three nest sites are known to exist all within Chobham Common, a nature reserve managed by Surrey Wildlife Trust. Understanding peak activity periods of foraging will help develop survey methods that more efficient, reliable and valid. Foraging activity was observed from 08:00–20:00. Foraging activity, number of workers leaving nest in 15 minutes, and prey returned to nest was recorded. Foraging activity closely followed soil temperature and to a less extent the air temperature, with the highest frequency of ants observed leaving the nest when the soil temperature was close to the peak for the day (14:00hrs). Plant material accounted for 15.8% of all food returned to the nests. The most common prey was Hemiptera, accounting for over 12%, closely followed by Hymenoptera and Coleoptera. Sugar bait stations offered a good estimate of foraging activity, a foraging area of approximately 500m^2 was found. Aggression bioassays were used to determine colony identities of two adjacent nests, and suggest they are closely related, supporting the belief they were produced by colony fission. *Dipoena tristis* (Theridiidae) was a regular predator at the nests. Results from study are used to suggest implications for survey methods.

Introduction

We are now approaching what scientists have named “The Sixth Extinction”. Animal and Plant species are disappearing at an astonishing rate, only seen five times in all of Earth’s history (Leakey & Lewin 1995). Although documentation of large vertebrate species features as a dominating force in media and even scientific literature, most of these extinctions are predicted to be that of insects (Dunn, 2005). Such a bias to studying vertebrate animals as they are more charismatic is a form of taxonomic chauvinism, invertebrates they have received less research funding and interest throughout history (Leather, 2009). It is however the invertebrate extinctions that may be most damaging to ecosystems, as they are often the foundations of them (Dunn. 2005) Invertebrates offer a diverse range of ecological system functions rendering them incredibly important to the local ecosystem, local extinctions are therefore as important as global ones. The UK has seen huge number of extinctions over the last century, many invertebrates have been lost most of which can be attributed to human activity (Shirt, 1987) Butterflies have been given the most attention in the invertebrate world and a massive decline in butterflies has been witnessed within the UK (Thomas *et al* 2004). There is very little known of all the other invertebrates with regards to their status, countless species could be in threat that we are unaware of. It is often only when a species becomes incredibly rare that attention is drawn and it is often too late.

Ants are considered an incredibly important taxa to ecological functioning acting as herbivores, predators, involved in soil aeration and important in plant seed dispersal mechanisms. They can be regarded as ecosystem engineers (Folgarait, 1998). One ant species has already been lost from Britain – the Black-backed meadow ant (*Formica pratensis*) (Shirt, 1987). Eight further species are at risk of being lost and have been placed on the Biological Action Plan (BAP) to prevent this. The Red-barbed ant (*Formica rufibarbis*) is arguably the rarest animal in Britain (Pontin, 2005) and is likely to be the next ant species to disappear from British shores if a concerted effort is not made for its conservation.

Formica rufibarbis

This species is a member of the Formicidae family of ants which has a further 8 representatives in the UK. It is distinguishable from the close relatives *Formica cunicularia* and *Formica sanguinea* by the presence of two barb like hairs on the prothorax which has led to the common name Red-barbed ant. Full identification keys can be found in Skinner & Allen (1996). Little is known of its breeding biology when compared to the intensely studied relative *Formica rufa*. Nests have been shown to include one or more queens and contain up to a thousand workers (Czechowski *et al.* 2002). The nuptial flights within the UK occur in June-July and nests are usually dormant from October through to April (Donisthorpe, 1927; Pontin, 2005). It is a thermophilic species preferring bare ground in full sun. Nests are often located underneath stones which increase the microclimate, achieving higher surface temperatures than that of the surroundings (Pontin 2005).

Workers forage singly for a diverse array of invertebrate prey (Donisthorpe, 1927: Jones, 2009) and have been noted to harvest honey dew from aphids where it is available (Pontin, 2005).

Distribution and status.

Formica rufibarbis is classed as a common species within continental Europe ranging across the Palearctic and is present in southern and central Europe as far north as 62 degrees latitude and spreads into Asia minor and Caucasus (UK BAP, 1998). There is however no data supporting a global population trend of the species (Gammans, 2008). Within the UK it is a very rare species, historically only living within the southern parts of the British Isles mainly in Surrey, along with a population in the Isles of Scilly (Donisthorpe 1927, Pontin 2005). In the early 20th Century *F. rufibarbis* was evident in large areas of heathland of South-east England consisting of many nests (Donisthorpe, 1927). Presently only three nests are known, all of which are within the nature reserve Chobham Common managed by The Surrey Wildlife Trust.

Causes for decline.

Formica rufibarbis is a specialist of sandy heathland (Pontin,2005), an endangered habitat itself, designated a BAP priority habitat in Britain. Sadly only 16% of Britain's heathland from 1900s remains (English Nature, 2002). Poor management of Britain's remaining heathland adds to the decline of this species. Grazing being removed from the remaining areas of heath led to increased vegetation succession. The increased cover of heather, gorse and bracken removed the favourable bare ground limiting the nest locations further (Pontin, 2005).

Conservation management

General heathland management is beneficial to the conservation of *F. rufibarbis* as well as a variety of other warmth loving invertebrates such as Solitary bees (Buckland, 2007). Grazing is beneficial to plant biodiversity and can increase percentage of bare soil however as populations are incredibly low the potential risk due to trampling is a real threat to the species' survival (Buckland, 2007; Pontin, 2002). Therefore scrape creation is one of the main tools used in Chobham Common where bare soil is created or banks are constructed to increase south facing slopes (Pontin, 2002). In 2008 twenty *Formica rufibarbis* colonies were released at Chobham Common. Queens were released with 'nest chambers' (small plastic box with entrance tube) with a minimum of ten workers along and a small reserve of fish eggs for food to maximise survivorship (Gammans, 2008). It is evident that a number of the sites failed and *Lasius niger* has colonised some locations and potentially killed some queens (Gammans, 2008).

Ants generally forage within a limited thermal range (Garcia-Perez *et al.* 1994) understanding this range is important information when predicting peak times of activity in order to achieve reliable surveying of foraging presence. Temperature has been shown to be a very strong factor in determining an ant's competitive ability and each species has what can be regarded as having a temperature niche (Hölldobler & Wilson, 1990). As this is very important for conservation of the species it is important to understand the temperature envelope that is most beneficial for the species. Jones (2009) found a maximum activity level in soil temperatures of approximately 30°C and activity falls at 50°C from the Isles of Scilly population. We expect this to be similar in the English population however no studies have focused on this, or the thermal qualities of nest sites themselves. In this study the aim is to understand the foraging ecology of the remaining nests of *Formica rufibarbis* on Chobham Common. There is also no data with regards to the prey that is taken by the English population. Jones (2009) found an adaptive prey selection in the Isles of Scilly population showing a positive quality for the conservation of the species. Understanding the foraging area of the remaining nests will develop an idea of areas to be searched when surveying for the released nests. Foraging distance has been stated at 10m (Jones, 2009) to 20m (Hölldobler & Wilson, 1990) from the nest entrance however this may be misleading information, which may not be applicable to the English population. Understanding peak activity periods of foraging will also lead to procedures that will lead to the most efficient survey methods, which is very important for such a rare species.

Aims

- Develop a temperature envelope under which the Chobham common nests are most active.
- Establish an estimate of the foraging distance and area of *Formica rufibarbis.*
- Note the main species of ants that interact with *F. rufibarbis* and determine if competition is evident.
- Compare the temperature of the release sites with that of the known nest sites, testing assumption that they are of similar value.
- Record prey taken by the nests noting any trends.
- Observe any predator species of the ants or interesting interspecies interactions.

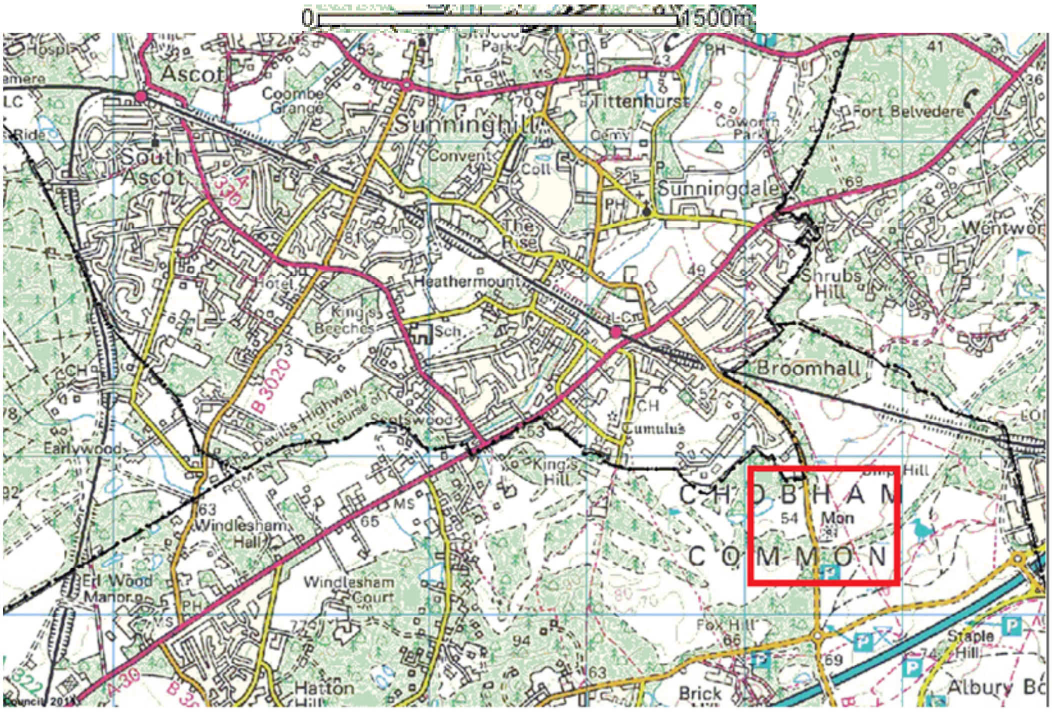
From the results of this study suggestions to survey methods for *Formica rufibarbis* on Chobham Common will be made. Further work that could be conducted will also be discussed.

Methods

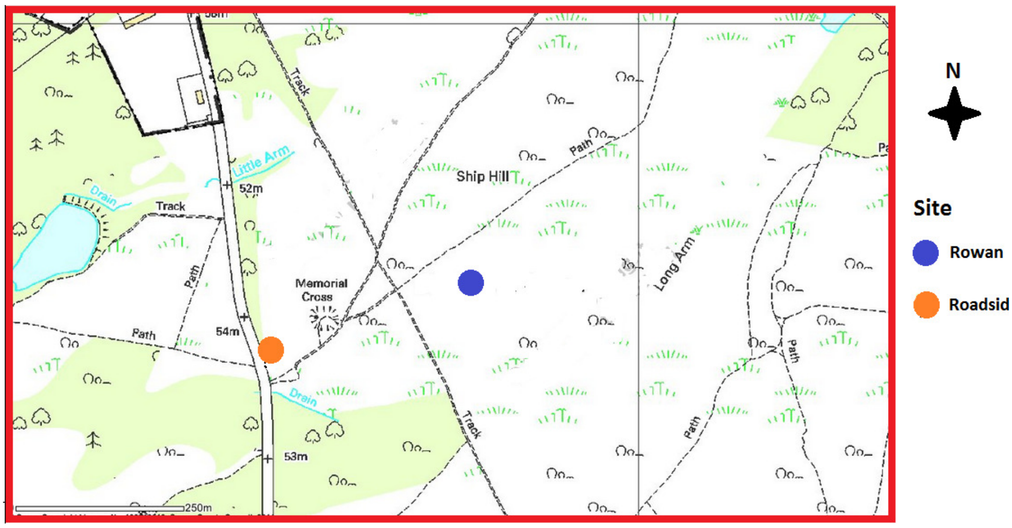
Studying such a rare species provides issues for the researcher, normal methods such as pitfall trapping, marking of individual workers or excavating of nests is not possible as this would significantly increase mortality rate (Billick, 1999). As there are only three English nests remaining any work that would increase mortality was avoided.

Study Site

The study was conducted in Chobham Common, Surrey (+51° 22’50", -0° 36’56”) located in South-east England from June-August 2011. The two known *Formica rufibarbis* nest sites in England are within 300m of each other and for the purpose of this study are named ‘Roadside’, due to a road to the West of the nests and ‘Rowan’, due to presents of a rowan tree to the East of the nest. The location of each is shown in Map 2.



Map 1. Location of Study site within Chobham Common, Surrey.



Map 2. Location of the two nest sites, Rowan and Roadside, within Chobham Common.

Roadside

(Grid reference SU 96440 65533)

This *F. rufibarbis* nest site is the newest discovery dating from 2008. It is a site of once overgrown gorse (*Ulex europeaus)* that had been cut back and a bund created. Dominant grass species were *Molinia caerulea* and *Agrostis curtisii,* Heather was also present however in much less frequency (*Erica cinerea, Calluna vulgaris* and *Erica tetralix*). Gorse, *Ulex minor,* was present. *Ulex europeaus* were up to a height of 1.4m. Birch saplings were scattered around the site with heights of approximately 1m. The B383 (Chobham Road) ran north-south to the west of the site. The two nest entrances were identified and named A and B in May 2011. Nest entrances A1, A2 were discovered June 2011 both of which showed to have connections to A. B1 was discovered August 2011 with a connection to B.

Photo 1. South facing view of Roadside site. B383 is to the west.

Photo 2. North facing view of Roadside site showing the areas of thicker vegetation. Nest locations are shown.

Rowan

(Grid reference SU 96701 65625)

This site is home for an established *F. rufibarbis* nest, its existence has been known for over 10 years (Pontin, 2002). The site has been subject to heathland management to increase areas of bare soil by turfing which would prevent vegetation succession that would shade out the species (Pontin, 2002). The nest site has been managed by the addition of stone slabs near the entrance to restrict both vegetation growth in the area and allow a higher temperature of the site.

Photo 3. Nest entrance at Rowan site. The coin in the centre of picture is a British ten pence piece.

Grasses *Molinia caerulea* and *Agrostis curtisii* dominate the areas where scrape has not occurred and vegetation covers 100% of ground at heights exceeding 30cm. Heather, mainly *Erica cinerea* and gorse (*Ulex minor* and *Ulex europeas)* also occurs across the site. A small patch of Birch trees is to the east of the site and one lone Rowan tree stands 8 m from the nest to the East.

Experimental procedure.

Foraging activity

Each nest entrance was observed from 08:00–20:00 for 3 days. Thermochrons (DS1921G-F) were placed close to the nest entrances approximately 1cm beneath the surface. Direct temperature readings were not possible as they would have disturbed the colony. A circular ring of string was suspended at with a diameter of 15cm around the nest. Numbers of ants leaving the string circle were recorded for 15 minutes at the start of each hour. Ants that did not leave the string were not counted as they as they taken as being involved in nest maintenance rather than foraging. A thermochron was also placed 20cm above the soil surface recording temperature when the study occurred. All thermochrons recorded temperatures every 30 minutes. Air temperatures were not recorded by the thermochron due to a technical fault. All air temperatures were taken from the nearby Woking weather station. (Met-Office, 2011)

Prey return.

All prey returned to the nest within 15minutes was recorded at the same time period as the foraging activity study was conducted. Recording for prey and activity was separated by 15 minutes as it is impossible to simultaneously record both sets of information accurately. Prey was identified to nearest possible taxonomic division. If prey could not be identified a digital photo was taken allowing later scrutiny, if this still did not help the item was labelled as unknown.

Bait station study

A 40m x 40m grid of bait stations spaced 4m apart was placed around the nest locations. Each bait station consisted of a $1cm^3$ piece of cotton wool soaked with 2ml of 20% sugar solution (w:v) resting on a 2cm x 4cm piece of card. Preliminary data showed that ants show no aversion to walking on the card, this aided ant identification. Recordings of number and species of ant present were taken after 1, 1.5, 2, 2.5 hour duration after placing down the bait stations. It took approximately 20 minutes to place down bait stations and the same time to record all ants present. Recordings were only taken on fair weather days with air temperatures exceeding 15°C. All data collection was taken between the hours of 12:00 and 15:00, as preliminary data showed this was the peak period of activity for the nests.

Thermal comparison of Nest and Release sites.

Thermochrons were positioned at release sites on Chobham common to develop an understanding of the soil surface temperature of these locations. Release sites were marked with slate and thermochrons placed in close proximity to these. Again the thermochrons were placed adjacent to release sites and 1cm beneath the soil, recording temperatures half-hourly for 16 days. Location of release site is shown in Map 1. Thermochrons used in foraging activity study were used to gather thermal data from the nests.

Photo 4. Example of release site slate. Red circle shows the position of the thermochron.

Nest aggression bioassays

The procedure was developed from that described in Carlin & Hölldobler (1986). Aggression crosses were taken from all the known active nest entrances (A, A2, B, & C) and all possible crosses performed. Foraging ants were taken as they left the entrance. One ant from each nest was placed within a Petri dish lined with clean filter paper. Ants were given 30 seconds to calm from the initial disturbance of being handled, after which time the behaviour was monitored for 2 minutes. The behaviour was scored according to the most aggressive act observed. After the assay was performed the ants were returned to the nest they were removed from. Each cross combination was repeated 10 times.

Table 1. Scoring responses of the aggression bioassay of ant pairings, taken from Carlin & Hölldobler (1986).

Score	
0	Casual tolerance; huddle together; allogroom; food exchange.
1	Initial jerk back, the tolerance; initial or weak avoidance.
2	Intense antennation ("investigation"); rapid mutual antennation; jerk back at each encounter; strong open-mandible threat.
3	Strong avoidance or flight; light mandible-mandible nipping ("nibbling"); aggressive regurgitation ("spitting fight"); stand atop.
4	Repeated, rapid forward-and-back jerking with open mandibles; stilt-legged posture; "advance-retreat" ; carry
5	Strong mandible-mandible nipping ("sparring"); seize and drag; lunge (weak charge); nip antennae, body, limbs; chase; gaster forward to spray acid
6	Charge and attack; briefly lock together; prolonged biting/ spraying fight

Statistical Methods.

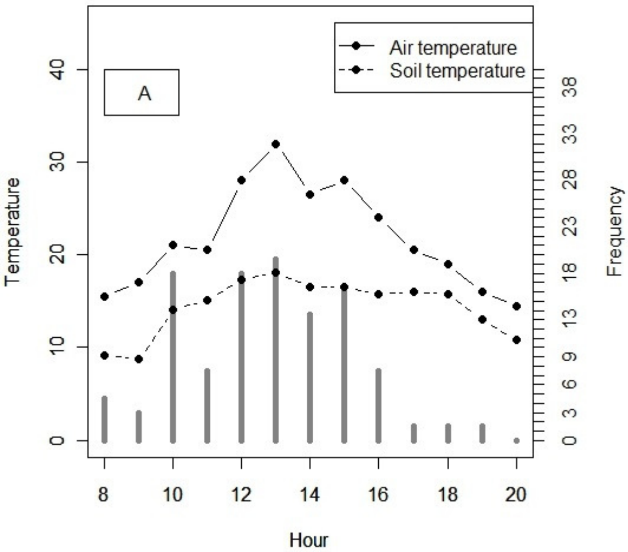
All statistical analyses, unless noted otherwise, were conducted in "R", version 2.11.0 (R Development core team, 2011)

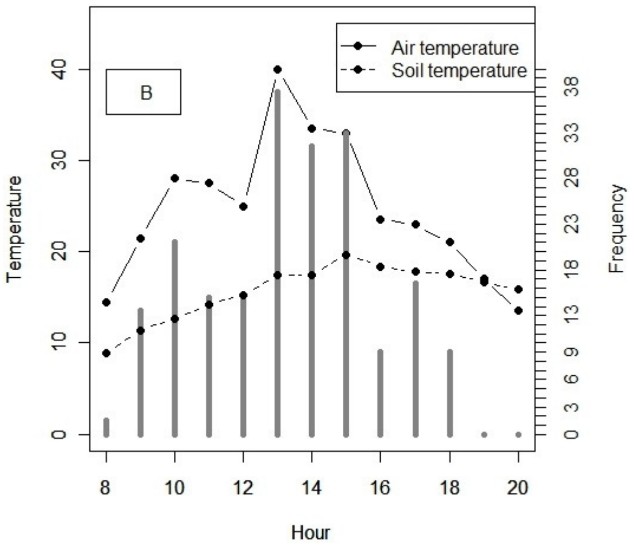
The comparison of prey frequencies returned back to the nests, A, B and C were compared using the likelihood of independence test (G-test). It was performed using the "R" package "Deducer" (Fellows, 2011). Thermal comparison of release and nest sites were analysed using repeated measures ANOVA where sites were taken as replicates and time of day as psuedoreplicates. Analysis was only conducted for temperatures from 08:00-20:00hrs as this data could be related to foraging activity. The Rayleigh test for uniformity, assessing the significance of the mean resultant direction of *F. rufibarbis* individuals at bait stations in relation to the nest. The test was performed in "R" using the package CircStats (Agostinelli, 2009). The C-score "checkerboardness" was used to determine whether there was a co-occurrence of species found at the individual bait stations or conversely if there was a significant checkerboard layout suggesting competition (Stone & Roberts, 1990). The simulation model EcoSim (Gotelli & Entsminger, 2001) was used to calculate the score. Observed data of species presence at each bait station was compared to null model Monte Carlo simulations of random species combinations constrained by totals from the matrix data. Five-thousand iterations were simulated and p values calculated from the location of the observed C-score on the normal distribution of simulated C-scores. See Stone & Roberts (1990) for a thorough explanation of the mathematical reasoning. Aggression bioassays were analysed using the non-parametric Mann-Whitney U Test comparing inter-intra scores of sites, (Rowan:Roadside) colonies (A:B) and nests (A2:A).

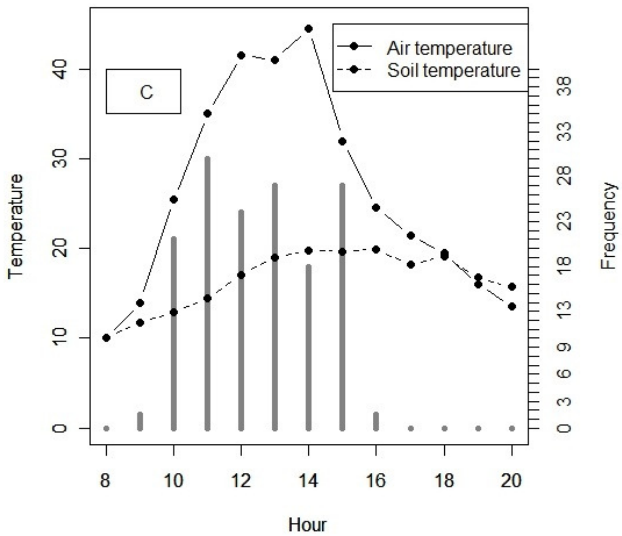
Results

Foraging Activity

Foraging activity, number of workers leaving nest in 15 minutes, closely followed the soil temperature and to a less extent the air temperature, with the highest frequency of ants observed leaving the nest when the soil temperature was close to the peak for the day (Figs 1-3)







Figures 1-3. Frequency of ants leaving the entrance of three study nests A,B and C within a 15 minute period against time of day. Corresponding air temperature and soil temperature is shown. Each graph shows results for one of the three day repeats. All temperatures are in degrees Celsius.

Numbers of ants leaving the nest increased with increasing temperature towards midday and then fell with the cooling in the afternoon. Temperature ranges were 9.5 - 57.5 ºC and 8.9 - 28.9 ºC for soil and air respectively, both of which showed a significant positive effect on ants leaving the nest. (Fig 4 & 5).

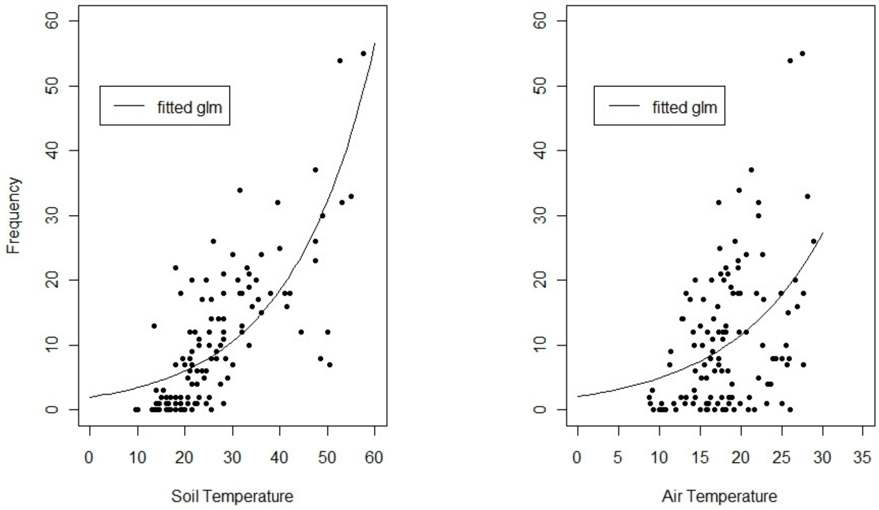


Figure 4 & 5. Activity as frequency of ants leaving in 15 minute period plotted against soil and air temperature. All temperatures are degrees Celsius. The data has been fitting with a quasipoisson GLM. Soil-temperature: y=exp(0.06x +0.68),T=3.71, d.f.=116, P<0.001, Air temperature : y=exp(0.09x + 0.73), T=10.83, d.f.=116,P<0.05.

Prey return

Table 2. Frequency of prey returned to nest entrance divided in taxonomic division from a total of 9 hours observation for each nest. Percentages are given in brackets.

Taxa	Nest							
	All		A		B		C	
Plant	37	(15.8)	9	(15.5)	9	(20.0)	19	(14.5)
Hemiptera	30	(12.8)	6	(10.3)	8	(17.8)	16	(12.2)
Hymenoptera	28	(12.0)	9	(15.5)	2	(4.4)	17	(13.0)
Coleoptera	28	(12.0)	9	(15.5)	5	(11.1)	14	(10.7)
Aracnida	21	(9.0)	2	(3.4)	1	(2.2)	18	(13.7)
Diptera	18	(7.7)	7	(12.1)	3	(6.7)	8	(6.1)
Isopoda	9	(3.8)	0	(0.0)	5	(11.1)	4	(3.1)
Orthoptera	9	(3.8)	2	(3.4)	4	(8.9)	3	(2.3)
Lepidoptera	8	(3.4)	3	(5.2)	0	(0.0)	5	(3.8)
Myriapoda	7	(3.0)	2	(3.4)	1	(2.2)	4	(3.1)
Annelid	7	(3.0)	0	(0.0)	1	(2.2)	6	(4.6)
Dermaptera	4	(1.7)	0	(0.0)	0	(0.0)	4	(3.1)
Mollusc	3	(1.3)	2	(3.4)	0	(0.0)	1	(0.8)
Unidentified	25	(10.7)	7	(12.1)	6	(13.3)	12	(9.2)
Total	234		58		45		131	

The most common prey type for the three nests was Hemiptera, accounting for over 12% of all prey observed being brought back to the nests, closely followed by Hymenoptera and Coleoptera. Arachnids was next most common however this result was mostly influenced by nest C where arachnids accounted for 13.7% of the returned food items but this was less than 4% for nests A and B. Plant material accounted for 15.8% of all food bought back to nest and this percentage was typical for all three nests. Diptera accounted for only 6.7% and 6.1% for nests B and C respectively however this value was nearly twice as high at 12.1% for nest A. The proportions of prey return from the different taxonomic divisions was not found to be significantly different between the three nests (G-test, G=29.17, d.f.=26, P=0.3).

Bait Station study

Roadside

The grid was limited in its extension to the West due to the road (B493). The majority of *Formica rufibarbis* were found in a southerly direction from the nest site, towards the road. Although over 5 species of ant were found at the site, it was rare for more than 2 species to be present at a bait station. *Formica fusca* was found at 32 of the 61 bait stations, proving to be a common ant species at the site, monopolising 11 of these. Co-occurrence of ant species at bait stations was not different to that that would be expected to chance (C-Score = 0.717, p = 0.10, N.S.). *Formica rufibarbis* was present at a total of 25 bait stations indicating a foraging area of 400m^2.

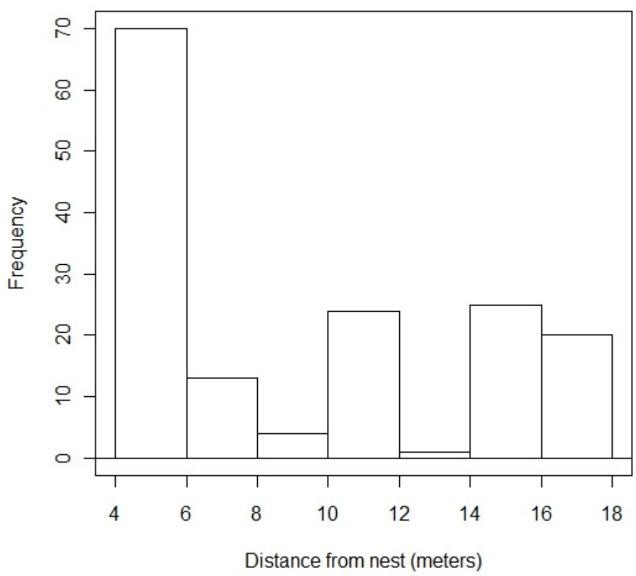
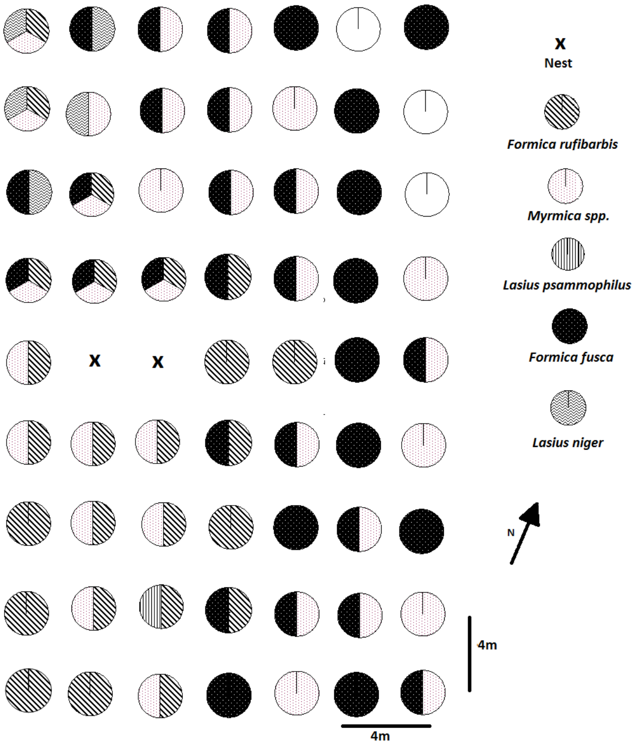
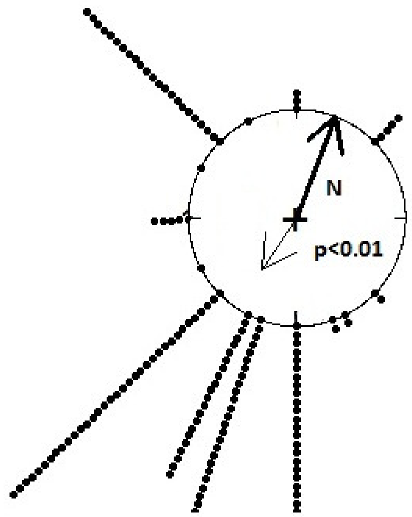


Figure 6 Frequency of *Formica rufibarbis* at Roadside bait stations in relation to the distance from the nest.

Formica rufibarbis workers were found up to 17.9m from the nest, the majority being 4-5m from nest. The mean distance was 9.5m. Figure 8 shows a histogram to represent the distance from nest data for the Roadside





Figures 7 & 8. *Above Left*: Schematic of Roadside bait stations showing presence of ant species *Above right*: Circular plot showing distribution of direction of *Formica rufibarbis* measured from Roadside nest site. The mean vector of distribution is shown by with thin line arrow. (Rayleigh test, n=163, rho=0.571 p<0.01)

Rowan.

Formica rufibarbis was not found at bait stations clustered in any particular direction relative to the nest. *Lasius niger* was the most common species of ant found at bait stations, showing presence at nearly half (39/81). Many of which were shared with *F. rufibarbis*. *Lasius niger* also showed a high competitive nature monopolising 9 of the stations. A total of 32 bait stations showed *F. rufibarbis* presence accounting for a foraging area of 512m^2. The furthest a worker was found from the nest was 17.9m, with the mean distance being 9.6m.

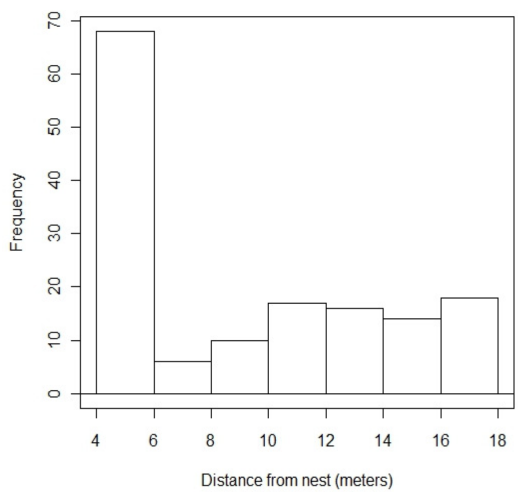
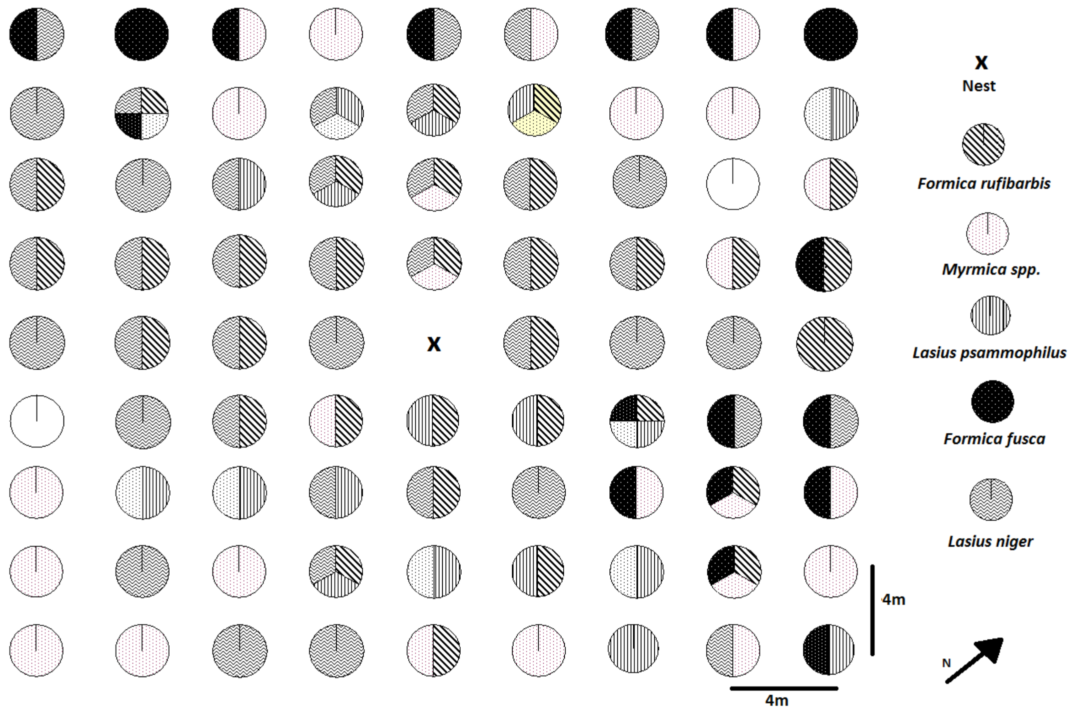
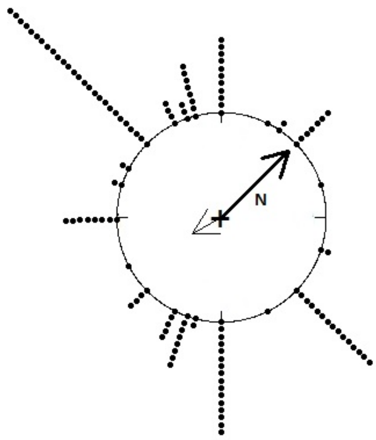


Figure 9 Frequency of *Formica rufibarbis* found a bait stations in relation to the distance from the Rowan nest.





Above: Figure 10. Schematic Road site bait stations showing presence of ant species.

Left: Figure 11. Circular plot showing distribution of direction of *Formica rufibarbis* measured from Rowan nest sites. The mean vector of distribution is shown by with thin line arrow. (Rayleigh test, n=142, rho=1.405, P=0.21, N.S.)

The co-occurrence of species at bait station was found to be lower than that expected by chance, supporting the extent of competition of species at this site. (C-score=1.322, p<0.001).

The mean number of *F. rufibarbis* counted at the bait stations increased with time (Fig. 12) however no significant difference was found (Kruskal Wallis, K=5.70, df = 3, p-value = 0.128). The mean number of bait stations with a *F. rufibarbis* present varied very little over time (Kruskal Wallis, K=1.14, df = 3, p-value = 0.767).

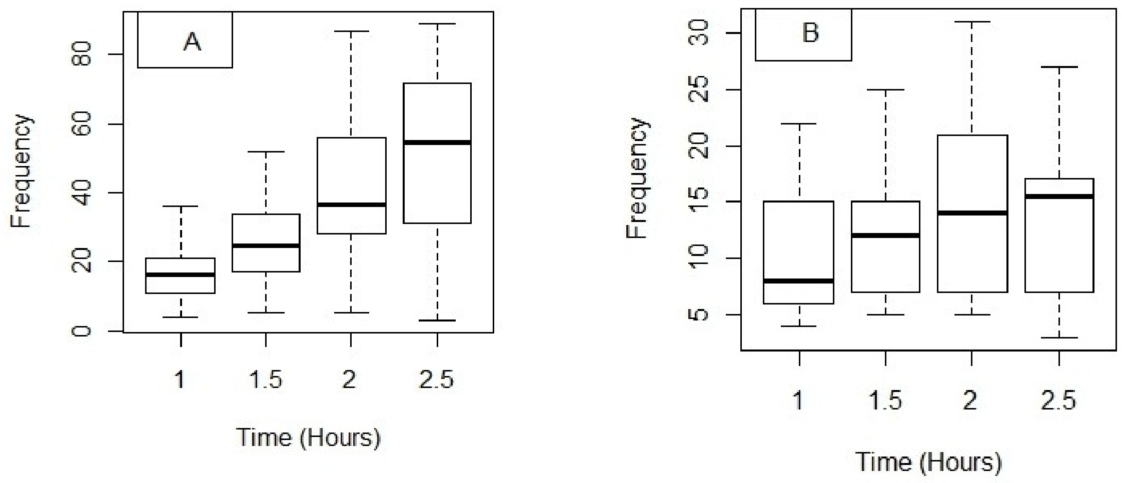


Figure 12. A- Frequency of *Formica rufibarbis* found at bait stations for different durations after placement. B- Frequency of bait stations with *Formica rufibarbis* present for difference durations after placement.

Thermal comparison of Nest and Release sites.

The pattern of temperature over a day is very similar between the nest sites and the release sites. Peak soil temperature is reached at 14:00hrs where the largest difference is seen, at this time average temperatures are 22.8 °C and 19.9 °C for the nest and release sites respectively. Although there was an apparent difference in the means of the two sites it was not found to be significant (Repeated measures ANOVA, n=9, F=1.19, P=0.303) between 08:00 and 20:00 when *F.rufibarbis* is likely to be active.

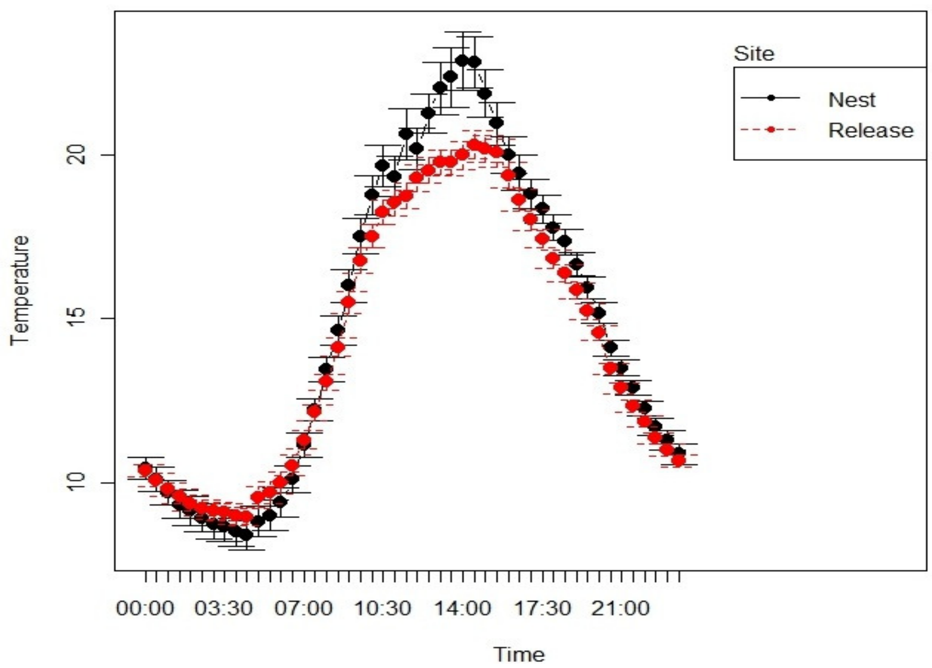


Figure 10. Mean temperatures (±S.E, °C) for nest sites (n=3) and release sites (n=7) recorded half hourly each day over 16 days. (05/06/2011 – 20/06/2011)

Aggression bioassays.

Aggression bioassays between the two sites Roadside and Rowan created the highest aggressive response averaging a 4 on the scale, significantly higher than the control (intra-site bioassays). Inter colony crosses between nests A and B score a much lower score when mixed averaging just 2, this score was consistently higher than the control. The lowest average score was observed between the inter nest crosses between A1 and A. This was not seen to be significantly different from that of the control.

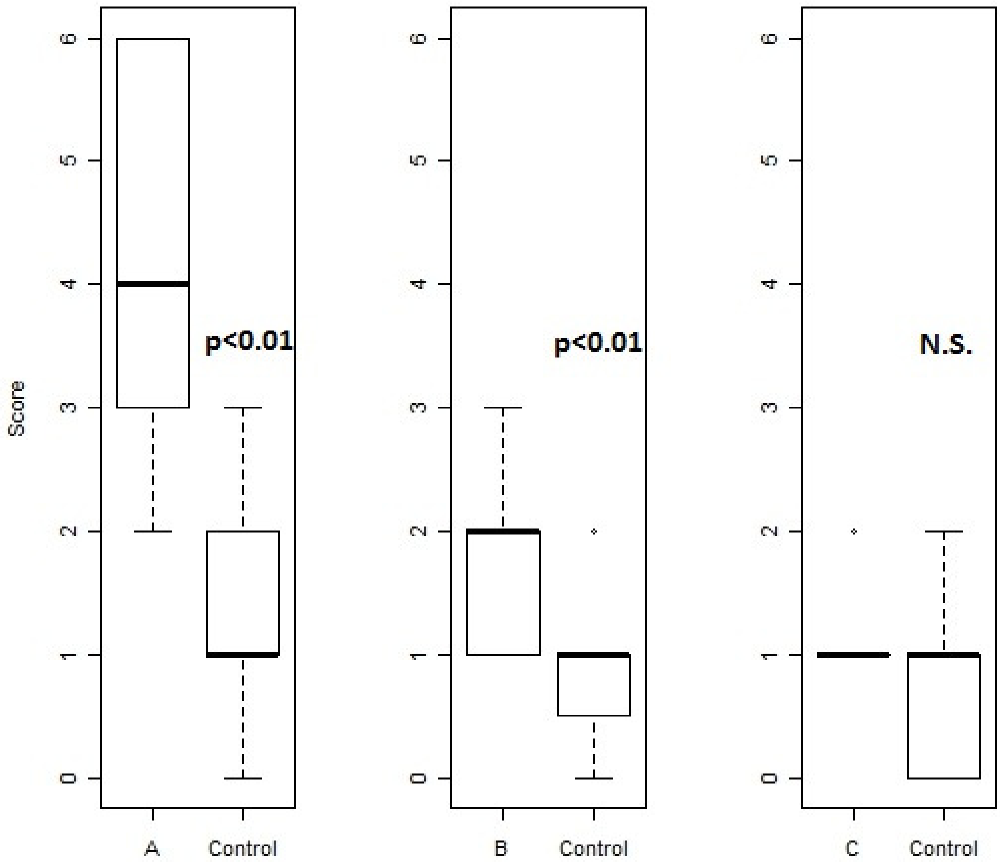


Figure 11. Boxplots showing the results of aggression bioassay tests and corresponding controls. A – mixes between nest sites, Road:Rowen (n=30, control n=70 Mann-Whitney U Test: M=57.16, d.f.=1), B – mixes between colonies, Colony A: Colony B (n=20, control n=40, Mann-Whitney U Test: M=656, d.f.=1), C – mixes within Colonies, A:A2 (n=10, control n=20, Mann-Whitney U Test: M=124, d.f.=1).

Observations

Workers were observed milking and tending to an aphid colony on a young gorse plant near to nest A throughout study period. Two queens were witnessed climbing the grass around nest C at 14.00hrs on July 14th 2011 at 13.00hrs. this suggests a nuptial flight had occurred close to this date. Activity of nests A and B was checked the same day but no queens were witnessed.

New nest entrances.

A1 - While conducting the study of foraging activity on Nest A the carrying of pupae into the entrance was witnessed. This first led to the discovery of a new nest entrance used by the colony on 22nd June 2011. The workers were followed back to their origin where a new nest entrance was confirmed 2.7m North of nest A's location in dense short cut grass. It was along a trail likely to have been created by a rodent. By 14th July 2011 it appeared the nest was abandoned, no activity was witnessed after this date. The entrance degraded over time and was barely visible by 21st August 2011.

A2 - Ant activity was observed 1.3m to the East of nest A and a new entrance was identified. The connection to nest A was confirmed when pupae originating from A were carried to this new nest.

B1 - When observing nest B ants leaving were witnessed carrying pupae. Following the workers led to the discovery of a new nest entrance 3m to the North at the base of a gorse stump in bare ground of soil and woodchip. This entrance was discovered 21st August 2011.

Predation.

When conducting the foraging study it became apparent that the obligate ant mugging spider, *Dipoena tristis* (Theridiidae), was often present at the entrances. Out of the 9 days monitoring nest activity the spider was present for 8 of these times. Three individuals were observed present at nest C on one occasion. They were very successful at catching ants around the entrances; with one spider taking 4 individuals in one day. A time of 3 hours was recorded from capture to complete consumption of one worker. The crab spider *Xysticus cristatus*w was also noted feeding on *F. rufibarbis.*

Above: **Photo 5.** *Dipoena tristis* feeding on a worker, found at nest C.

Right: **Photo 5**. *Xysticus cristatus* feeding on worker, found at nest A.

Agelena labyrinthica is very common at both the Rowan and Roadside sites, webs were often within a meter of the entrances. *F .rufibarbis* was never witnessed falling victim to this species. Workers walked onto the web sheet on two occasions and fought off the attack from this species. It should be noted however that this was an observation was not of an adult *A. abyrinthica*. Webs of *A. labyrinthica* were checked for remains of *Formica rufibarbis* but predation by this species was not confirmed.

Discussion

Temperature/ Foraging activity relationship

This study shows how the activity of the nests, number of ants leaving per unit time, increases with increasing temperature. This relationship can only be used to describe the temperatures that were experienced in this study, a range of 9.5 - 57.5°C and 8.9 - 28.9°C for soil and air respectively. We cannot extrapolate from this data that this relationship will continue into higher temperatures. The GLM shows how the activity begins to rise after approximately 15°C. Ants have been shown to have a thermal niche where they show activity, yet above and below this niche activity will cease and potentially the individuals will die (Hölldobler & Wilson, 1990). Jones (2009) and Nielson (1981) found that soil temperatures above 35°C actually led to a reduction in activity. The data from this study showed activity continuing to rise at soil temperatures up to 50°C showing a much higher temperature tolerance. It could be that the English population of *F. rufibarbis* is actually adapted to living in a warmer environment than that of the Isles of Scilly or Denmark (Nielson, 1981). The mean high temperatures given in June for Isles and Scilly and Woking are 17 and 20 respectively (Met-Office, 2011). This higher temperature experienced by the English *F. rufibarbis* colonies may have led to a better adaptation to warmer soil temperatures. A small increase in air temperature is likely to suggest a larger soil temperature increase due to the nonlinear relationship (Green *et al.* 1984). Further studies would need to be conducted to test this theory. Perhaps monitoring colonies produced by queens originating from the Isles of Scilly to see if they have a lower temperature tolerance. Equally the difference may be a result of discrepancies of soil temperature recording. Although methods of recording temperature were identical between the studies it is plausible that soil erosion brought upon by rain caused the thermochrons to come closer to the surface. Soil depth is crucial for determining temperature (Oliver *et al.* 1987). If the depth of the thermochrons was less in this study a higher temperature would be recorded. To combat this discrepancy in soil temperature recorded a more accurate method to measure actual surface temperature such as an infrared thermometer could be used.

Although the GLM shows the activity continuing to rise, the data suggests that the peak activity temperature was nearly reached in the observations taken for soil temperatures above 50°C. Looking at the plots of frequency of ants leaving and temperature you can see that there are 4 points near 50°C that are of considerably lower than that of the calculated GLM. Ants are known to have a minimum and maximum activity temperature (Hölldobler & Wilson, 1990). To ascertain what the peak temperature is it is recommended that more readings of foraging activity should be taken incorporating more temperatures exceeding 50°C. It would be beneficial to take data from a warmer month, August for example. This however could not be used to compare activity from another month as number of ants within a colony may alter within a season (Hölldobler & Wilson, 1990). The activity level would have to be standardised to account for changes in number of foragers in a colony over the season.

It could be argued that as temperature and time of day follow a specific pattern and it is merely an artefact that activity follows temperature. A diurnal rhythm based on time which peaks around midday might just be followed. This argument however is not likely to be a valid one. Time of sunrise changes by nearly 2 hours over the spring and summer season showing that time is not a reliable measure to determining activity. Hunt (1974) showed how activity rhythms of ants are controlled more by temperature and environmental factors rather than diurnal time rhythms. A unimodal activity rhythm can be turned into a bimodal one by shading a nest around midday (Hunt, 1974) the reduction in temperature this caused significantly reduced foraging activity supporting a temperature related activity pattern in ants.

Prey return.

Prey return studies retrieved disappointing results in terms of number of observations. Only a total of 58 prey items were witnessed being returned to nest A in a total of 9 hours observation. This result could be due to the fact that there were other active nest entrances for this colony (A1 and A2). Workers could potentially be returning food items to these entrances, causing a reduction in number of observed food entering nest entrance A. Nest B also provided disappointing numbers of prey returned, it is possible that an entrance was concealed underneath the gorse. The second nest entrance of colony B was not found until August but it may be possible that it was present throughout the whole season. The bait station study does support the presence of *F. rufibarbis* to the north of the nest B, where the new entrance B1 was found. (Fig. 5), so we cannot rule out its presence at the time the bait station study was conducted.

Bait station study

Previous work on the Isles of Scilly population has noted that *Formica fusca* does not occur in the same localities as *F. rufibarbis* (Brian, 1964). These two species do occur together in Chobham Common. The C-score for the Rowan site showed a level of competition for bait stations between the ant species. It is highly likely that these are competitive species limiting each other's local distribution. This would limit *F. rufibarbis* to foraging in the higher temperature soil types created by bare soil, where it has the advantage over *F .fusca* and others*. Formica fusca* has been shown not to prefer bare soil (Brian, 1964; Buckland, 2007), this was seen in the grid station study where most of the *F. fusca* was found to the North and east of the Roadside nests where covers of Gorse and *Molinia spp.* formed a high percentage of ground cover.

The maximum distance from the nest a worker was found was 17.9m for both sites which is similar to the distance noted in Hölldobler & Wilson, (1990) however much larger than the distance noted from Jones (2009), but this study only use anecdotal evidence to come to this conclusion. Although there seems to be a higher frequency of ants foraging at 4-5m distance from the nest this is likely to be a misleading result. Ants leaving the nest entrance to forage are likely to come in contact with the bait stations at 4m and start feeding rather than foraging at further distances. The numbers observed are evenly distributed suggesting that they forage uniformly with respect to distance from the nest. This study could be improved by placing a ring of sugar bait stations immediately outside of the nest. This would prevent new foragers leaving the nest influencing the frequencies at the 4m bait station. This would get a realistic snap-shot of where the foraging workers are in relation to the nest entrance. The area covered by *F. rufibarbis* was 512^2m in the Rowan site and $400m^2$ for the Roadside site. The grid station however was likely not to have sampled all the foraging area of the Roadside site as the road acted as a barrier to the ants. It is likely that the ants carried on further South-west along the path. Assuming that the Roadside will have equal foraging area to the Rowan site foraging could continue for a further 16m. It would be interesting to focus a study on the prey retrieval in *Formica rufibarbis.* The grid bait station study could not distinguish between members of nest A or B in the roadside site. An investigation that sadly could not be completed in this study due to time constraints could be easily conducted to see whether nest A and B had overlapping foraging areas. Following a method described in Bouley *et al.* (2007) a detailed foraging territory map can be constructed. Dividing the study area into distinct grids and observing each grid for *F. rufibarbis* activity, workers can be offered a distinct packet of food (usually tuna). The worker could then be followed back to the entrance, giving foraging territories and area for specific colonies. This could also provide information on whether one nest entrance was preferred for returning prey to in a colony. This data could retrieve very important information for this species of great conservation importance. If strong territories are observed it suggests that two colonies forage antagonistically, this would suggest perhaps they be relocated for a mutual benefit, or that any further reintroductions of *F. rufibarbis* be spaced further apart to prevent competitive in foraging between conspecifics.

The total number of bait stations with the species presence did not increase over time. This information suggests that sugar bait stations will provide a reliable measure of *F. rufibarbis* foraging activity of a location within an hour of being placed out, little more information is likely to be gained from an increasing sampling time to 2. 5 hours. Sugar bait stations were used to measure the foraging activity in this study as studies showed it provides a good bait to capture a range of ant species (Way *et al.* 1997). Given further time a study focusing on preferred bait station would have been conducted to ascertain the best possible bait to survey *Formica rufibarbis.* Tuna is regularly used for such predatory species as *F. rufibarbis* however this might not actually be beneficial to surveying this species. *Formica rufibarbis* has good eyesight and likely forages using sight when compared to other ant species (Donisthorpe, 1927). Using odoriferous Tuna may favour ant species that use olfactory cues preferentially which would not be beneficial. It may be that different coloured bait stations could have important in creating a better bait station for *F. rufibarbis.* A simple study could be conducted to ascertain the best colour increasing accuracy of survey technique of *F. rufibarbis* such information would be incredibly important considering the post-release colonies have yet to be found.

Colony identities

From this study it seems that the Roadside site consists of two distinct colonies. The new entrances A1, A2 and B1 are proven to be of the same colonies of A and B respectively, due to the exchange of pupae and workers between them. However the lack of interaction observed between nests A and B does not qualify them as distinct colonies. Gammans (2008) suggests the two nests are satellite nests however no work has really focused on confirming this. The aggression bioassays have shown that members of the two nests A and B have a significantly different aggressive behavioural response to each other. Such behavioural responses support a self/non-self distinction that workers from different colonies are making (Carlin, 1989). Although the mean response is not of an especially aggressive manner they are significantly different to that of control B-B, A-A and A2-A2 crosses. An interesting finding is the significant difference of inter and intra aggression of the sites. Inter site bioassays (A, A2 and B crosses with C) produced high levels of aggression, the only bioassays that produced scores above 3. Nest colony identification in ants has been shown to be based from genetic and environmental factors creating differing hydrocarbons coated on the workers (Jackson & Morgan, 1993). Although it has been shown that diet (Liang & Silverman, 2000) and nesting material (Bos *et al.,* 2011) is important in the ability for colonies to identify self from non-self, this has been shown not to be the case for *F. rufibarbis*. Van Zwedon (2011) shows through cross fostering studies that it is heritable factors that influences the hydrocarbons that are involved in nest mate recognition in *F. rufibarbis* which impacts on aggressive bioassays. This being known the results of this study suggests the close relatedness of nests A and B and not that of C. The distinguishing of inter and intra site colony aggression is suggestive that colony formation has been due to fission (Dahbi *et al.* 1996) which supports what is suggested by Gammans (2008). These results are far from conclusive as there is far more research to be conducted on nest mate recognition in ants. It would be interesting to test aggression bioassays on the Isles of Scilly's population to determine factors of nest aggression, to decipher what determines aggressive response and whether it consistently corresponds to relatedness or merely nest proximately. This could be related to DNA studies which are currently ongoing (Gammans, 2008). Caution should be taken with the interpretation of these studies. Experimenter bias is a problem regular featured in scientific research (Sheldrake, 1998), this study was conducted by a single experimenter and results may have been influenced by an expectation of result. To remove this possible criticism I suggest a repeat using a double blind experimental set up using a naive person grading ant aggression bioassays.

Conclusion and Implications

Sugar bait stations offered a good estimate of foraging activity for *Formica rufibarbis,* strong attraction to the bait stations was witnessed. Foraging distance was shown not to exceed 17.9m with the mean distance approximately 10m. I would therefore suggest that bait stations for survey work of *F.rufibarbis* should be placed in grid like formation with distances not exceeding 18m. Preferably it should be 10m to increase chance of falling in the foraging range of the workers. If the distance exceeded 18m survey work could easily miss the forging range of a nest. The Roadside nest showed increased foraging activity alongside the road, this suggests survey work should be focused near to roads. Time showed little effect on number of bait stations with *F.rufibarbis* present between 1hour and 2.5 hours. It seems that survey work involving bait stations would produce reliable data on the presence/absence within an hour after the bait stations had been set out, little benefit to increasing duration of surveying was indicated. Competition between ant species is evident at the sites. *Lasius niger* has high competitive effect, monopolising bait stations frequently. *Formica fusca* is rarely found in conjunction with *Formica rufibarbis* they likely exclude each other locally.

A significant relationship was found for the foraging activity of the nests for air and soil temperatures. It suggests that air temperatures increasing up to 29°C have a beneficial impact; survey work should be taken at these warmer temperatures to increase chance of finding a forager. For Chobham common the highest soil temperature on average is at 14.00hrs which suggests the peak activity for the nests. Temperatures above those experienced within this study may not follow the same pattern of increased activity. A reduction in activity is predicted for soil temperatures exceeding 50°C and air temperature above 29°C.

Nests A and B are likely to have been formed through fission as the low aggression bioassays suggests a high level of relatedness when compared to nest C. The different aggressive response between nests A and B suggest they are independent colonies but the score is low enough to suggest they have overlapping territories. More work needs to be completed to ascertain if this assumption is correct.

As heritable factors have been shown to be involved in nest identification unrelated queens are likely to lead to highly aggressive colonies, suggesting queens from different blood lines should not be released close to each other.

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