Surveying abundance and stand type associations of *Formica aquilonia* and *F. lugubris* (Hymenoptera: Formicidae) nest mounds over an extensive area: Trialing a novel method

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Abstract. Red wood ants are ecologically important members of woodland communities, and some species are of conservation concern. They occur commonly only in certain habitats in Britain, but there is limited knowledge of their numbers and distribution. This study provided baseline information at a key locality (Abernethy Forest, 37 km²) in the central Highlands of Scotland and trialed a new method of surveying red wood ant density and stand type associations: a distance sampling line transect survey of nests. This method is efficient because it allows an observer to quickly survey a large area either side of transect lines, without having to assume that all nests are detected. Instead, data collected on the distance of nests from the line are used to estimate probability of detection and the effective transect width, using the free software “Distance”. Surveys took place in August and September 2003 along a total of 71.2 km of parallel, equally-spaced transects. One hundred and forty-four red wood ant nests were located, comprising 89 *F. aquilonia* (Yarrow, 1955) and 55 *F. lugubris* (Zetterstedt, 1838) nests. Estimated densities were 1.13 nests per hectare (95% CI 0.74–1.73) for *F. aquilonia* and 0.83 nests per hectare (95% CI 0.32–2.17) for *F. lugubris*. These translated to total estimated nest numbers of 4,200 (95% CI 2,700–6,400) and 3,100 (95% CI 1,200–8,100), respectively, for the whole forest. Indices of stand selection indicated that *F. aquilonia* had some positive association with old-growth and *F. lugubris* with younger stands (stem exclusion stage). No nests were found in areas that had been clear-felled, and ploughed and planted in the 1970s–1990s. The pattern of stand type association and hence distribution of *F. aquilonia* and *F. lugubris* may be due to the differing ability to disperse (*F. lugubris* is the faster disperser) and compete (*F. aquilonia* is competitively superior). We recommend using line transect sampling for extensive surveys of ants that construct nest mounds to estimate abundance and stand type association.

INTRODUCTION

Red wood ants play an important role in the ecology of woodland ecosystems by virtue of their large numbers and biomass. Their predation on defoliating insects benefits trees (Karhu & Neuvonen, 1998) and shrubs (Atlegrim, 2005), and is sufficient to cause shifts in relative abundance of different insect guilds, such as higher densities of aphids and leaf miners, which are not preyed upon (Fowler & MacGarvin, 1985). Other insects form symbiotic relationships with ants, including myrmecophilous beetles (Päivinen et al., 2004). Ant distribution may also determine where birds feed through competition for common food, and nest through avoidance of ants (Haemy, 1992, 1999).

In the northern pinewoods of Europe, red wood ants are the most prominent group of ants, though in Britain, there is concern for the status of some species, including *Formica aquilonia* (Yarrow 1955), which is nationally scarce (Anon., 1999; Hughes, 2005). In Scotland, several red wood ant species inhabit ancient native pinewoods, itself a habitat of high conservation status (Anon., 1995). The ancient native Scots pine *Pinus sylvestris* (Linnaeus, 1753) forests of Abernethy, Glenmore and Rothiemur-
as distance sampling (Buckland et al., 2001); the methods
are used routinely to estimate population size and density
of mammals and birds, but not insects. Although mapping
all nests in a study area is clearly the gold standard, it is
extremely labour intensive and hence rarely possible in
practice for large areas of interest. Line transect surveys
are more efficient than both mapping and plot sampling
approaches because there is no need to exhaustively
search an area to ensure that no nests are missed. Instead,
observers can move relatively quickly along a set of sys-
tematic or randomly placed transect lines, recording the
distance to all mounds detected either side of the line, and
this information is used during analysis to estimate the
detection probability or, equivalently, effective transect
width. We provide the first assessment of the numbers
and distribution of nest mounds of red wood ants in Aber-
nethy Forest, holding the largest remnant of ancient
native pinewood in Scotland (Mason et al., 2004). This
method also allowed us to determine associations with
stand types. The survey focused on the two commoner
species: *F. aquilonia* and *F. lugubris*. *F. aquilonia* is a
polygynous species (many queens in one colony) and
polydomous (colonies with cooperative nests) (Rosengren
& Pamilo, 1983). It is generally associated with large old
forests (Punttila, 1996). By contrast, *F. lugubris* is a
monogynous (a single queen in a nest) and monodomous
species generally associated with younger forest stands
(Punttila, 1996).

**STUDY AREA AND METHODS**

Abernethy Forest (57°15′N, 3°40′W) lies within Strathspey
on the northern slopes of the Cairngorm Mountains, in the cen-
tral Highlands of Scotland, 200–500 m above sea level. The
stand types within the native pinewood were old open
woodland, high-crown woodland and wooded bogs. The forest
also had plantation woodland, largely planted after 1930, com-
prising pole-stage, thicket and pre-thicket. Almost all the trees
were Scots pine, whilst the shrub layer comprised juniper *Jun-
iperus communis* (Linnaeus, 1753), heather *Calluna vulgaris*
(Linnaeus, 1753), bilberry *Vaccinium myrtillus* (Linnaeus,
1753) and cowberry *V. vitis-idaea* (Linnaeus, 1753) (Summers
et al., 1997).

Ant mounds were surveyed between 22 August and 9 Sep-
tember 2003 using a line transect protocol (Buckland et al.,
2001). The single observer (KMB) traversed seventeen parallel
transects, running east-west across the forest and 500 m apart,
and totalling 71.2 km within 37.2 km² of woodland (Fig. 1).
Visibility was good on all 13 days of the survey. A sighting
compass and Global Positioning System were used to maintain
direction along transects. Once a mound was located, its bearing
and radial distance from the transect was measured. This
allowed calculation of the perpendicular distance from the tran-
sect. The size (width at the base and height) of each nest, as
seen from the transect, was measured, so that any differences in
detectability due to nest size could be examined. There were no
cases of partially connected mounds, so all could be regarded as
separate mounds. When the transect line was left to measure a
mound, no other mounds were searched for until returning to the
line. The stand types in which the mounds occurred were also
noted. Different stand types were initially given descriptive
categories, but were later combined into four functional stand
types of woodland development: stand initiation, stem
exclusion, understory re-initiation and old-growth (Oliver &
Larson, 1996) (Table 1). Areas without trees, including clear-
fells, remained as a separate category. A sample of five ants was
collected from each nest mound and stored dry for later identifi-
cation (Collingwood, 1979).
Estimation of density and population size from resulting data was performed using the software Distance, version 6.0 release 2 (Thomas et al., 2010). Preliminary detection function models were fitted, and the data were truncated (ignored beyond a set distance) so that probability of detection at the truncation distance was approximately 0.15, as recommended by Buckland et al. (2001). As a check, we also investigated the effect of using other truncation distances. Various detection function models were then fitted, and the model with lowest Akaike’s information criterion (AIC) value was selected. Candidate detection function models had either half-normal or hazard rate key functions with cosine or simple polynomial series adjustments, and also included ant species, stand type (the four categories) and nest size (half-width, as seen from the transect, multiplied by height, i.e. approximate area visible from a side view) as covariates affecting the scale parameter of the key function (Marques et al., 2004). The final selected detection function model was then used in conjunction with the encounter rate (number of nests per km line length surveyed) to estimate density by ant species. Variance was estimated using estimator O2 of Fewster et al. (2009) for encounter rate variance, which gives a more reliable and usually lower estimate for systematic parallel lines than the standard method (Fewster et al., 2009).

To quantify stand type associations for each species, Jacobs’ selection index (Jacobs, 1974) was calculated by stand type category. The index \(D\) was derived from the proportion of stand type available \(p\) relative to all stand types, and the proportion of the species observed within that stand type \(r\): \(D = \frac{(r-p)(r+p-2p)}{(r-p)(r+p-2p)}\). Values can potentially range between 1 (full association) and –1 (full avoidance). It was likely that the detection of mounds varied among stand types because it was easier to see below the crowns of larger trees than smaller ones. Therefore, in this comparison, the data were truncated so that only those nests within 20 m of the transect were selected. Confidence intervals on the selection indices were calculated using a nonparametric bootstrap, resampling transect lines with replacement 1000 times. This analysis was performed using custom-written code in the statistical software R (R Development Core Team, 2010).

RESULTS

One hundred and forty-four nest mounds of red wood ants were located along the transects. These comprised 89 \textit{F. aquilonia} mounds and 55 \textit{F. lugubris} (Fig. 2). The mean height of \textit{F. aquilonia} mounds was 44 cm (range 15–110 cm) and the mean width 96 cm (range 24–200 cm). The dimensions of \textit{F. lugubris} mounds were similar: the mean height was 46 cm (range 13–99 cm) and mean width 95 cm (range 27–188 cm). However, mound height varied significantly amongst stand types, being highest for both species in stem exclusion stands (57 and 52 cm for \textit{F. aquilonia} and \textit{F. lugubris}, respectively), and shortest in old-growth stands (40 and 35 cm for \textit{F. aquilonia} and \textit{F. lugubris}, respectively) \((F = 3.59, P = 0.017\) for \textit{F. aquilonia} and \(F = 3.05, P = 0.037\) for \textit{F. lugubris}). Nevertheless, as the apparent side view area for the two species was practically the same, we anticipated that the detectability of the mounds would be similar in the detection function modeling.

The distribution of distances of detected nests from the transect line showed a spike at small distances, coupled with long right tail: the median distance was 5 m but the mean was 9 m and the maximum 72 m. The selected truncation distance was 4 m, leaving 66 observations, and at this truncation, the AIC best-detection function was a half-normal model with no adjustments or covariates (Fig. 3). The goodness of fit test indicated a satisfactory half-normal model with no adjustments or covariates (Fig. 3). The goodness of fit test indicated a satisfactory model (Kolmogorov-Smirnov test, \(D_r = 0.10, P = 0.52\)). The effective half-width strip was 2.4 m (CV 10.5%). This provided a density estimate of 1.13 mounds per hectare (95% CI 0.74–1.73) for \textit{F. aquilonia} and 0.83 (95% CI 0.32–2.17) for \textit{F. lugubris}. These density estimates translated to total numbers of 4,200 (95% CI 2,700–6,400) and 3,100 (95% CI 1,200–8,100) mounds, respectively, for the whole forest (Table 2).

To check the importance of the truncation point, we undertook confirmatory analyses at truncation distances of 2, 10, 20 and 25 m, and found that density estimates

![Fig. 2. The distribution of Formica aquilonia and F. lugubris nests along the transects at Abernethy Forest.](Image)
some association for old-growth woodland, although the 95% confidence interval in the latter case included zero, so no definitive statement can be made. *Formica lugubris* mounds were found in areas of wooded bog, pole stage woodland and thickets. They did not occur in old open woodland and clear-felled areas. Indices for selection (Table 3) indicated that *F. lugubris* were associated with stands at stem exclusion, with evidence of avoidance of understorey re-initiation and old-growth, although 95% confidence interval in the former just included zero.

**DISCUSSION**

**Estimation of nest mound abundance**

We used a line transect sampling method to investigate red wood ant nest mound density for the first time in this study. This method has several general assumptions which we were able to meet; (1) that all target subjects (nest mounds) on the transect line are detected, (2) that the subjects do not respond to the observer prior to detection, (3) that encounters with the subjects are independent, and (4) that measurements of angles and distances are accurate (Buckland et al., 2001). In several respects, ant nest mounds are ideally suited to transect sampling because they do not move, thereby fulfilling assumptions 1–3. Training, and the use of measurement aids ensures assumption 4 is fulfilled.

Our study found red wood ant densities of 1.13 nests per hectare for *F. aquilonia* and 0.83 for *F. lugubris*. These nest mound densities were similar to those found in Finland, where Puntila (1996) reported 0.8 and 1.7 per hectare for *F. aquilonia*, and 1.9 and 0.1 per hectare for *F. lugubris* in young and old conifer forests, respectively. Values around 1.2 mounds per hectare appear to be typical for total forest areas in Finland (Wuorenrinne, 1975), though specific areas can have higher densities; 4–6 nests per hectare for *F. aquilonia* (Vepsäläinen & Wuorenrinne, 1978) and 2.6–2.9 nests per hectare for all red wood ants (Domisch et al., 2005). Exceptional densities of over 100 red wood ant nests per hectare can sometimes occur (Vepsäläinen & Wuorenrinne, 1978). By contrast, Breen (1979) found a much lower density (0.13

**TABLE 2.** Sample size (within truncation distance of 4 m), estimated density (number per hectare), abundance and coefficient of variation (CV) of *F. aquilonia* and *F. lugubris* nest mounds at Abernethy Forest, based on the line transect analysis. 95% CIs are given in brackets.

<table>
<thead>
<tr>
<th>Sample size</th>
<th>Density</th>
<th>Abundance</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Both species</td>
<td>66</td>
<td>1.96 (1.19–3.22)</td>
<td>7,300 (4,400–12,000)</td>
</tr>
<tr>
<td><em>F. aquilonia</em></td>
<td>38</td>
<td>1.13 (0.74–1.73)</td>
<td>4,200 (2,700–6,400)</td>
</tr>
<tr>
<td><em>F. lugubris</em></td>
<td>28</td>
<td>0.83 (0.32–2.17)</td>
<td>3,100 (1,200–8,100)</td>
</tr>
</tbody>
</table>

**TABLE 3.** Indices of stand association for *F. aquilonia* and *F. lugubris* nest mounds at Abernethy Forest. Positive values indicate association and negative values avoidance. 95% CIs are given in brackets. Open areas were not included because no nests were found there.

<table>
<thead>
<tr>
<th>Stand type</th>
<th><em>F. aquilonia</em></th>
<th>Sample size</th>
<th><em>F. lugubris</em></th>
<th>Sample size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stand initiation</td>
<td>0.13 (0.59, 0.58)</td>
<td>14</td>
<td>0.06 (0.39, 0.58)</td>
<td>8</td>
</tr>
<tr>
<td>Stem exclusion</td>
<td>–0.20 (0.73, 0.29)</td>
<td>13</td>
<td>0.42 (0.03, 0.69)</td>
<td>21</td>
</tr>
<tr>
<td>Understorey re-initiation</td>
<td>–0.62 (–1.00, –0.21)</td>
<td>4</td>
<td>–0.20 (–0.76, 0.01)</td>
<td>8</td>
</tr>
<tr>
<td>Old-growth</td>
<td>0.37 (–0.19, 0.77)</td>
<td>46</td>
<td>–0.33 (–0.77, –0.07)</td>
<td>12</td>
</tr>
</tbody>
</table>
nests per ha) of *F. lugubris* in a conifer plantation in Ireland.

Variation in density of nest mounds amongst stand types and transects occurred in our study, and therefore the design of future studies should be carefully considered. The coefficients of variation for nest densities were high for both species in our study (Table 2), particularly for *F. lugubris*, leading to wide confidence intervals. Uncertainty in line transect estimates comes from two sources: estimating the effective strip width, and mean encounter rate (number of nests per transect line divided by line length). In this study, most of the variation was due to the encounter rate component – i.e., due to differences in encounter rate along the different transects. For *F. aquilonia*, 75.2% of the overall variance in the density estimates came from this source, whilst for *F. lugubris* it was 95.2%. This percentage would have been even higher had we not used systematic design coupled with the use of the newly-developed overlapping post-stratification encounter rate variance estimator (O2) of Fewster et al. (2009), which helped minimize the effect of the observed north-sound gradient in nest density. Encounter rate variation could have been substantially reduced by post-stratifying by stand type and hence negating the effect of between-stand type variation in density, but sample size was insufficient. We therefore recommend the systematic parallel design for future surveys, but suggest a higher survey effort together with regular recording of the stand type along the line, so that it will be possible to post-stratify estimation by stand type.

Truncating at the distance at which probability of detection was approximately 0.15 led to a truncation point of 4 m being chosen and around 54% of the data being excluded from the density analysis. Confirmatory analyses at a wide range of truncation distances gave less than 10% variation in estimated density, indicating that the results are robust to the choice of truncation, as is typical for line transect studies (Buckland et al., 2001). At the largest truncation distance (25 m), stand type and nest size were both selected as covariates in the final detection function model. It was not surprising that these factors became more important at larger distances as nest mounds are more obvious when larger or in, for example, more open stand types. Sample sizes of detections are also larger, enabling AIC to select more parameter-rich models. Surprisingly perhaps, at larger truncation distances, the variance of the overall density estimates was larger than at the 4 m truncation distance chosen. This was because at larger distances, the spike in detections in the first few metres followed by a long tail in detections at larger distances became a prominent feature, and the hazard rate detection function, which is able to fit such a pattern, became the key function selected as the best model by AIC. The intercept of this spiked function was estimated with considerable uncertainty, leading to a greatly increased variance in estimated effective strip width and hence an increase in overall variance. Hence, we believe our decision to follow Buckland et al.’s (2001) recommendation to truncate at a distance at which probability of detection was approximately 0.15 led to more reliable inferences about population density. In our case, this approach led to quite extreme truncation (over 50% of the observations) and resulted in a truncation distance of 4 m. Future surveys should focus search effort more into the region close to the line (up to approximately four metres). This will reduce the spike in observations within 0.5 m of the line (Fig. 3), and lead to greater precision in detection function estimation, as well as less sensitivity of results (such as variance) to the chosen truncation distance. We suggest that observers can, for example, leave the transect line to search behind nearby obstacles (such as large trees) so long as the position of the line is known so that distance from nests to line can be accurately measured, and no nests on the line are missed.

**Stand type associations**

Our transect survey method was able to identify spatial segregation of the two species, with *F. aquilonia* in the southern part of Abernethy Forest and *F. lugubris* more in the central area (Fig. 2). These distributions were associated with particular stand types; *F. aquilonia* tending to associate with the old-growth stands found mainly in the south and *F. lugubris* with younger more central stands. These associations concur with the results of a previous study (Punttila, 1996). *Formica aquilonia* is usually thought to be tolerant of the low light conditions found in young stands (Punttila, 1996). However, we suggest that *F. aquilonia*’s tolerance of low light conditions (Punttila, 1996) was not observed because the forest floor in the old-growth woodland at Abernethy Forest has greater irradiance than understory re-initiation and stem exclusion stands (Parlane et al., 2006). It appears that long-term stability in the old-growth woodland was more important than light conditions.

The patterns of stand type association we found can be attributed to differences in each species’ ability to disperse, colonise and compete (Savolainen et al., 1989). Some queens are also dependent on acceptance into a nest of a species of the sub-genus Serviformica to establish a colony because they cannot raise broods on their own (Dekoninck et al., 2010). *Formica lugubris* disperses further than *F. aquilonia* because of its higher frequency of alate-producing nests, resulting in mating flights and the establishment of a new nest elsewhere, whereas *F. aquilonia* queens often do not have a nuptial flight and may return to the original colony (Rosengren & Pamilo, 1983). Expansion is through the slow process of budding of nests (Punttila, 1996). Therefore, *F. lugubris* manages to colonise young forest before *F. aquilonia*, but because *F. aquilonia* is competitively superior, it tends to monopolise older forest, presumably by out-competing any *F. lugubris* (Vepsäläinen et al., 2000). However, in an experiment, Gibb & Johansson (2011) did not observe a change in the species composition of other ant species when *F. aquilonia* was removed.

The current age structure of the forest and hence the assemblage of ant species present in Abernethy Forest may be attributed to past human use. Much of the southern half of Abernethy Forest, dominated by *F. aqui-
is ancient native pinewood, which has been relatively undisturbed since 1869 when crofters, who would have grazed livestock in the woods, were removed and the area became a sporting estate (Summers et al., 1997, 2008). Even during the crofting era, the silviculture was a shelterwood system, so that some old trees were always retained as seed trees (Steven & Carlisle, 1959). Selective felling plus browsing by livestock and the increasing red deer Cervus elaphus (Lönnberg, 1906) population, encouraged for sport shooting, would have inhibited tree regeneration and kept the woodland open and light levels reaching the forest floor high. The northern part of Abernethy Forest, with higher densities of F. lugubris, has had a history of more aggressive forestry management. Much was clear-felled during the early 19th century but replanted from 1830 (Summers et al., 1997, 2008). There were also stand replacing fires, followed either by ploughing and replanting or leaving the sites to regenerate naturally. Thus, in the northern half of Abernethy we tended to find F. lugubris, some F. aquilonia, or no ant nests at all.

Our study suggests that some forest management may have hindered red wood ant persistence. No ant nests were found in clear-cut areas in the northern part of Abernethy (Mondhuié) that was recently (1997–2001) cleared of thickets of lodgepole pine Pinus contorta (Douglas ex Loudon, 1838). Clear-felling is detrimental to red wood ant populations because worker ants lose visual cues for orientation and food sources such as aphids in trees (Rosengren & Pamilo, 1978; Vepsäläinen & Wuorenrinne, 1978). It is likely too that prior to clear-felling, the thickets of lodgepole pine would have provided poor habitat for ants owing to the low light levels on the forest floor. Supporting this hypothesis, there was evidence of some ants establishing nests on edges of thickets, including along a cleared ride under a pylon line, where more light was available.

Land preparation techniques also appeared to be associated with low nest mound densities. Nests were notably absent in areas that had been ploughed and planted with Scots pines in the 1970s and 1980s (Clais an Eich, Bogelchynack, Rynettin, Bognacruie and North Abernethy; Fig. 1) (Summers et al., 1997). These areas once had old-growth woodland, so we assume they had F. aquilonia nests. However, clear-felling and ploughing probably eliminated any nests (Punttila et al., 1994; Domisch et al., 2005). To date, over 30 years after the disturbance, recolonisation by the F. aquilonia and F. lugubris has been poor, though some F. exsecta (Nylander) nests occur in these areas, particularly in North Abernethy (RSPB, unpub. data).

Nest mounds also appeared to be absent from Tore Hill (Fig. 1), that had been burned accidentally in 1920 and then replanted in 1932 (Summers et al., 1997). The stands there are now at the understory re-initiation stage but are probably still too dense to allow sufficient light and warmth for establishment by ants (Skinner & Allen, 1996).

To maintain the abundance and diversity of red wood ants, we suggest forest managers need to ensure that the stand characteristics required by each species are provided. It is likely that there will be slow changes in the ant community associated with stand dynamics and previous land use of the forest (Dauber et al., 2006). Minimising the fragmentation of stands and ensuring that plantation woodland is contiguous with old-growth woodland will help the spread of ant species (Punttila, 1996; Hughes, 2005). A time-lag is expected before ants respond to landscape change (Rosenengren et al., 1979; Dauber et al., 2006). This is demonstrated by our finding that the areas that were ploughed have not even been colonised by F. lugubris after 30 years. This concurs with a Belgium study where 25 years was insufficient time for all woodland species to establish in new forests (Dekonnick et al., 2008). However, as the forest ages and becomes re-colonised by ants, it is likely that the ant community will gradually become to be dominated by F. aquilonia, until the next disturbance episode (Vepsäläinen & Savolainen, 1994; Punttila et al., 1994).

CONCLUSION

We suggest that the line transect survey method, based on distance sampling, used in this study was successful in determining red wood ant densities and illuminating stand type associations. However, careful consideration of truncation distances, and therefore the area focused on by observers, is required for future surveys. We recommend that land managers consider a similar methodology when aiming to identify the nest mound density and distribution of other red wood ant communities.

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