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The spatial distribution and environmental triggers of ant mating flights: using citizen-science data to reveal national patterns

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Abstract

Many ant species produce winged reproductive males and females that embark on mating flights. Previous research has shown substantial synchrony in flights between colonies and that weather influences phenology but these studies have been limited by sample size and spatiotemporal scale. Using citizen science, we gathered the largest ever dataset (>13,000 observations) on the location and timing of winged ant sightings over a three-year period across a broad spatial scale (the United Kingdom). In total, 88.5% of winged ants sampled were *Lasius niger*. Observations occurred from June to September with 97% occurring in July/August but exact temporal patterns differed substantially between years. As expected, observations within each year showed a small but significant northward/westward trend as summer progressed. However, the predicted spatiotemporal synchrony was far less apparent; observations were not significantly spatially clustered at national, regional or local scales. Nests in urban (vs. rural) areas and those associated with heat-retaining structures produced winged ants earlier. Local weather conditions rather than broad geographical or seasonal factors were shown to be critical in the timing of winged ant activity, presumably to optimize mate finding and to minimize energy consumption and predation. Temperature and wind speed, but not barometric pressure, were significant predictors of observations (positively and negatively, respectively); winged ants were only observed at temperatures >13 °C and wind-speeds <6.3 m^{-1}. All days with a mean daily temperature >25°C had observations. Intriguingly, changes in temperature and wind speed from the day before flight peaks were also significant. We conclude that: (1) spatiotemporal synchrony in flights is lower than previously thought for *L. niger*, (2) local temperature and

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wind are key predictors of flight phenology; and (3) ants appear able to determine, at least in a limited way, if weather is improving or deteriorating and adjust their behaviour accordingly.

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Introduction

Phenomena involving large numbers of animals acting more-or-less synchronously across a range of spatial scales are of considerable interest to both ecologists and the general public. Mass emergences or movements are valuable systems to investigate the temporal synchrony, spatial co-occurrence and triggers of significant biological events. Such phenomena may also create a short-lived abundance of prey, making them likely to be important drivers in trophic interactions within ecosystems. Well-known examples include the annual mass migration of wildebeest (*Connochaetes taurinus*) around the Serengeti (Berger 2004), the mass spawning of Atlantic horseshoe crabs (*Limulus*) on the eastern coast of the USA (Walls et al. 2002), the emergence of swarms of Chironomidae flies from Lake Victoria (Corbet 1958), and the appearance of adult periodical cicadas in the USA (*Magicicada*) in predictable 13- and 17-year cycles (Simon 1988).

Conceptually, mass biological phenomena such as these involve some element of temporal synchrony and spatial co-occurrence. However, when the patterns of such events are examined in detail, they frequently turn out to be less synchronized and less coordinated than previously thought. Horseshoe crabs, for example, spawn across lunar cycles and thus, although they might use the same beach, they do so at different times in the season (e.g. Smith et al. 2002), while there is considerable variation in the timing of East African wildebeest migration both within and between years (Boone et al. 2006).

Many ant species have nests that are founded by a single queen (the winged reproductive female caste) who has mated with one or more winged males during a mating flight. Multiple winged reproductive males and females are produced by ant nests in great

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numbers at certain times of the year (Hölldobler and Wilson 1990). In species such as *Lasius niger* (a species prevalent in this study – see Results), males aggregate *en masse* with females joining to mate ("male aggregation syndrome") (Hölldobler and Wilson 1990). In such species the emergence of winged reproductive individuals is apparently highly synchronized within and between nests often across large areas. Not only does this mass emergence facilitate out-breeding, it also acts as an anti-predation strategy through the dilution effect (e.g. Rubenstein 1978). However, despite longstanding scientific interest in ant mating flights (Huber 1820), our understanding of the synchronicity and coordination of mating flights, and of the environmental cues that trigger them, remains patchy. In part, that is because current datasets are often highly-localized spatially (e.g. Wilson 1957; do Nascimento et al. 2011; Kaspari et al. 2001) or highly-restricted temporally (e.g. Feitosa et al. 2016).

The lack of broad-scale data on ant mating flights spanning multiple years means that potential environmental triggers are not always clear. A number of climatic factors are likely to be important, for example, higher temperatures make flight more energy efficient, whereas high wind speed makes flying difficult or impossible. Recent rainfall can make digging the initial nest cavity easier (e.g. Staab & Kleineidam, 2014). Several studies have investigated the climatic conditions associated with mating flights, with some intriguing results, but such studies are limited by restricted spatiotemporal contexts. For example, Boomsma and Leusink (1981) studied mating flights across three consecutive years but predominately on a single island in the Netherlands. They concluded that time of day, relative humidity and global radiation were significant factors determining flight days for *Lasius niger*, *L. flavus* and *Myrmica rubra* (but not for *M. scabrinodis*). They also noted

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that mating flights did not occur at wind speeds >1.7 ms^{-1} at 2 m above the ground until part way through the season. At that point, mating flights occurred at higher velocities, indicating a shifting threshold based on reduced flight opportunities later in the season. Depa (2006) compared weather conditions on flight days versus non flight days for *Manica rubida* in Poland, concluding that flight days had significantly higher daytime temperatures than non-flight days, but that wind velocity and air pressure did not differ. However, these findings were based on the observation of just 5 flight days covering 113 reproductive females in a total area of 2,000 m^2. A study of the leafcutting ant *Atta vollenweideri* concluded that mating flights occur in the spring only after a cumulative precipitation of at least 64 mm in the last month before the first mating flight and only if temperatures rise above 26°C on the days following rainfall (Staab & Kleineidam, 2014). Finally, a study of mating flights of the harvester ant *Messor barbarus* across the Iberian Peninsula, based on 123 mating flight observations across 7 years, concluded that flights were associated with specific weather fronts, but only data from 2008-2009 were robust enough to analyse (Gomez and Abril 2012). Taken together, these studies suggest that weather conditions are likely to be important triggers for mating flights. However, further progress on characterizing these triggers will only be possible with data covering a far greater number of flights and encompassing both a wider geographical area and a longer time period than has been possible in previous studies.

Understanding the intrinsic and extrinsic triggers for mass events, such as flying ant emergence, is complex because spatiotemporal information on both the events themselves, and potential biological drivers, is needed across a wide spatial area and throughout the relevant season. To achieve this could require large numbers of observers to be present

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across the study area (which might be national or even transnational) over a considerable period of time. The discipline of citizen science, especially mass participation projects where scientists work in partnership with the public, has developed rapidly (e.g. Hart et al. 2012) and has already been used to investigate biogeographical patterns including the migration routes of butterflies (Howard and Davis 2009) and the population dynamics of birds (Wood et al. 2011). Since multiple citizen scientists can record examples of phenomena across wide geographical areas over a considerable period the approach has great potential for the study of mass biological events.

Here, we use a citizen science approach to overcome the typical limitations inherent in studying ant mating flights. Engaging the public to report ant mating flights proved to be a powerful way of studying this phenomenon at a national scale, throughout the entire flying season, and across multiple years, yielding >13,000 records between 2012 and 2014. We analyze this dataset to determine: (1) the temporal pattern of mating flights within and between years; (2) the spatial co-occurrence of mating flights at a range of scales from national to local; and (3) the environmental and climatic factors that trigger flights using additional Meteorological Office data. We hypothesise that ant mating flights will be aggregated temporally, at least at a local or regional scale if not nationally. We expect weather variables, especially temperature and wind speed, to be correlated with the timing of ant flights with flights being most likely to occur on warm, still days

Materials and Methods

Citizen Science Survey

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An online survey, promoted through the website of the Royal Society of Biology (RSB), was established in 2012 using the online survey tool *Survey Monkey*. Online information covering ant biology and mating flights, as well as photographs of winged males and females of various species was provided. The survey was open from the 1st June (day 1) to the 4th September (day 96) in 2012, 2013 and 2014.

Participants from the UK (excluding Northern Ireland) were asked to record observations of winged, ants, known colloquially in the UK as "flying ants", and provide the date and time of their observation, together with location as a street address, postcode, or latitude/longitude coordinates. Observations could be of individual winged ants, of large numbers emerging from a nest or of a mating flight involving multiple nests. In a second optional step, participants were able to record whether their observation was in a rural or urban area and provide details of the nest environment (such as under a pavement or within a greenhouse). Individual participants could record multiple observations within and between years. In total, 16,139 individual records were gathered throughout the 3-year period.

In all three years, the RSB issued press releases that were widely used by national, regional and local print and online news as well as broadcast media (radio and TV) to promote the study. Most UK national newspapers, BBC Online, and hundreds of local and regional papers published articles. A variety of online news sources also publicized the survey. The authors took part in more than 50 radio interviews on local and national networks over the three years of the project.

Ant species identification

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Participants from the first survey in 2012 who expressed an interest in becoming more involved with the study the following year were, in 2013, sent a small sample tube and pre-addressed, postage-paid envelope. This allowed them to return samples of winged ants collected and reported that year. Of the 1,200 tubes sent out, 436 were returned and the ants contained within them were identified to species using Identification Key II (female reproductive ants) and Key III (male ants) provided by Skinner and Allen (1996). In all cases of multiple ants within the same tube (ca. 50% of tubes), the ants were of the same species and were treated as a single record. A small number of tubes (N = 9) contained Diptera or other Hymenoptera, which were not identified.

Data cleaning

The survey data from the three different years were cleaned and standardized to produce one combined dataset. Any historical records (i.e. people recording an observation they had seen in previous years) were deleted, as were records outside the UK and incomplete records. A total of 2,745 records were removed during this data cleaning step, leaving 13,394 analysable records. Remaining records were standardized: dates were transformed from calendar format into an ordinal day number (1-96) and locations given as postcodes or addresses were transformed into latitude and longitude. Each record location was also assigned to the nearest UK Metrological Office weather station (N = 124).

Analysis of temporal patterns

Observations within each year were tested against a uniform distribution (i.e. one that assumes observations were equally probable on every day across the season) using a Chi-

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Square Goodness of Fit test to determine whether observations were non-uniformly distributed in time.

Spatial patterns: latitude and longitude

To analyze the effect of latitude and longitude, a generalized linear mixed model (GLMM) was used. The model contained day number as the response variable, latitude and longitude as fixed factors, and year as a random factor. The *glmer* function was used with estimations based on the restricted maximum likelihood (REML) method from the R package *lm4* (Bates et al. 2015). Significance was determined with Type II Wald Chi Square tests as implemented in the function *Anova* from the R Package *car* (Fox and Weisberg 2011). Given that the dependent variable was continuous, and after examining residual plots to ensure homoscedasticity, the Gaussian variance function with link identity was used. In addition to checking for homoscedasticity, the model was validated against the assumptions of normality, lack of multicollinearity, and autocorrelation (Thomas et al. 2013). Normality was considered to be within the limits acceptable for a GLMM to be robust, especially considering that the remaining skew was consistent between all groups (Glass et al. 1972). The variance inflation factor (VIF) was 1.143, substantially below the maximum of 10 recommended by Myers (1990), indicating the multicollinearity was within acceptable limits. Spatiotemporal autocorrelation was checked using the Durbin-Watson test with the underlying data formatted so records were ordered by time within location; this returned a value of 1.828 and was thus well within the accepted range of 1.5 and 2.5 (Field, 2000). The marginal coefficient of determination (R_m^2 summarizing variance explained by fixed factors) and the conditional coefficient of determination (R_c^2 summarizing variance

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explained by both fixed and random factors) were estimated following the method described by Nakagawa and Schielzeth (2013) using the R package *arm* (Gelman and Su 2014).

Spatial patterns: Geographical clustering

To investigate the degree of geographical clustering of winged ant observations across the UK, we calculated the Euclidean distance based on associated longitude and latitude coordinates. For each day with 2 or more individual observations, we calculated the mean actual Euclidean distance between these points (D_{actual}), which we then compared using a bootstrapping method to the mean Euclidean distance of 10,000 samples of the same number of randomly-drawn observations from the entire dataset for that year, which spanned 96 days from June to September (D_{mean}). In this way, the number of observations was the same for each comparison (e.g. if there were 14 observations on one day so D_{actual} was based on 14 data points and D_{mean} was also based on 10,000 samples of 14 data points, drawn randomly from the overall dataset). As distance measures were non-normal and actual distances showed large fluctuations on days with few observations, we recorded days where D_{actual} was lower than D_{mean} as "nearer" and tested for significance with a sign test, where the number of times the actual distances were nearer (N_{nearer}) was compared to the total number of comparisons (N_{total}). The same process was used in all three years, although the number of days with 2 or more observations, and that could thus be included in the test, differed between years.

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The same Euclidean distance bootstrapping method was applied to a subset of the data from the Greater London region, as defined by the area covered by the five weather stations coded as Greater London (Kenly, Northolt, Heathrow, Brixton, Bromley). This gave 1,958 observations (15% of all observations) across three years. Again, a sign test was used to test whether D_{actual} was lower than D_{mean} by quantifying the number of times the actual distances were nearer (N_{nearer}) relative to the total number of comparisons (N_{total}).

Some clustering of flights was apparent on specific days (see Figure 1). We thus selected 6 geographical areas from across the UK that were both widely geographically distributed and had a large number of records (>230 in all cases) to analyze this in greater depth. Each geographical area was a polygon centered on a UK Metrological Office weather station (mean distance to observations across polygons = 19.1km, SD = 4.0). All observations within the area covered by this polygon (i.e. all observations for which this weather station was the nearest geographically) were included (Table 1). Together the six selected areas accounted for 1167 observations (9%) across all years. We used a multivariate ordination-based framework based on principal components but extended by use of a bootstrapping approach to allow the significance of any differences between clusters of data points to be determined objectively (Catlin-Groves et al. 2009). The analysis was carried out in R using the procedure outlined by Stafford et al. (2012) with a 95% confidence interval and a bootstrap size of 10,000. The outcome of the analysis was a three dimensional plot comprising six spheres – one for each weather station – for each of the three study years. The spheres indicated ant flight distribution through time, with the centre of the sphere indicating the mean and the radius showing the 95% confidence radius around that mean. As with standard PCA plots, the

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resultant plot is in arbitrary units but here, because of bootstrapping, the distance between the spheres is representative of the extent of the difference between the observation distributions at that location. More importantly, overlap between spheres signifies that those distributions do not differ statistically ($p = 0.05$), whereas a gap indicates a significant difference. Thus, where spheres did not overlap, this was statistically-robust evidence that the temporal distribution of observations at the two locations those spheres represented were significantly different (for a more detailed description of the method, see Catlin-Groves et al. (2009) and Stafford et al. (2012)). We ran the analysis by setting up a distribution table of the frequencies of observations at each site within separate 3-day blocks. This subset was chosen such that the highest possible number of observations was included (99.4% in 2012, 99.6% in 2013 and 94.4% in 2014) while keeping the number of days per block as low as possible (note that the number of time periods was limited to 19 by the R function used: Stafford et al. 2012).

Environmental correlates with mating flights: habitat and microhabitat

The separate effects of habitat (urban or rural) and microhabitat (association with heat retaining structures) on the mean observation day were investigated with a GLMM using the approach described above. Observation day was the response variable. The first categorical fixed factor described habitat – whether the nest was in an urban location (1) or not (0). A second categorical fixed factor was used to quantify microhabitat – whether winged ants emerged from under stones, pavements or from compost heaps or greenhouses (i.e. a warm microhabitat; 1) or not (0). Both fixed effects were based on optional citizen science survey responses where participants could indicate the habitat and microhabitat

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status of their observation using check boxes. In addition to the two fixed factors of habitat and microhabitat, year and nearest weather station (to control for geographical effects) were entered as random factors. The model used the Gaussian family with link identity. All assumptions were tested as detailed above for the latitude/longitude model and were met (VIF = 1.013; Durbin-Watson = 1.836).

Environmental correlates with mating flights: climate

For each reported observation at a given location (coded 1) we created six additional records, one for each of the three preceding and three subsequent days (each coded 0). The weather conditions for each of these days were taken from the nearest weather station to that location as described above. To investigate the effect of environmental variables on the likelihood of observations occurring on a given day, we constructed a GLMM with a binary response variable describing observation days (1) and non-observation days (0). This approach has been used previously by Depa (2006) and Staab & Kleineidam (2014). Daily mean temperature (°C), wind speed (mph) and pressure (mbar) were added as fixed effects. It was not possible to include precipitation since this was given only as a monthly total and our analyses required daily data. For each of these parameters, one other fixed effect was added to describe the differences between that parameter (temperature, wind, pressure) relative to the previous day, thereby creating three "trend" factors. This approach is similar to that taken by Staab & Kleineidam (2014) who consider that the time span is biologically reasonable to trigger changes in ant behaviour. For each trend factor, the value could be positive (increase from previous day; 1), negative (decrease from previous day; -1) or, if the

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difference was less than one degree Celsius (temperature), 1 mph (wind speed) or 2 mbar (pressure), "no change" in which case it was assigned a value of zero. Using these threshold values, approximately a third of observations were assigned to each category. Weather station and year were added as random factors to allow for the effects of space and time. A full model including all second order interaction terms (excluding interactions between the primary weather factors and the associated trend factors) was built. A model simplification process was then used to remove non-significant terms using backwards elimination (starting with all predictor variables and removing the least significant first, then the next least significant and so on until all remaining variables were significant at $\alpha = 0.05$). Although backwards elimination based on significance values can have some drawbacks (Goodenough et al. 2012), in this case it was an effective way of fine-tuning the model. The final model was validated against the assumptions of heteroscedasticity and lack of multicollinearity as detailed above for the latitude/longitude model and were met (maximum VIF = 1.376; Durbin-Watson = 1.660). All average values are given as mean± std. error, N = sample size. All statistical tests were carried out with R version 3.0.2 (R Core Team 2013) and the critical significance level was 0.05.

Results

Ant species

Of the 436 ants returned using posted sample tubes in 2013, 386 (88.5%) were *Lasius niger*. Of the remaining 50 ants, 37 (8.5%) were *L. flavus*, 3 were *Myrmica lobicornis* (0.7%), 2 (0.5%) were *Formica candida*, 2 (0.5%) were *L. alienus* and the remaining 6 (1.4% in total)

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were single ants of *L. mixtus, L. brunneus, L. sabularum, L. fuliginosus, M. rubra* and *Tapinoma madeirense.*

Temporal patterns

Observations were not uniformly distributed in any year (actual distribution compared to a uniform distribution across 97 days: $\chi^2 = 54761$, d.f. = 96, $P < 0.001$ in 2012; $\chi^2 = 31882$, d.f. = 96, $P < 0.001$ in 2013; $\chi^2 = 39306$, d.f. = 96, $P < 0.001$ in 2014). For all years combined, there were more records submitted in July (64.6%) than in June (2.1%) or August (32.6%), with 0.7% of observations in September (2014 only). The timing of peaks in observations differed markedly between years (Fig. 1). The highest number of observations for each year was on 24th July 2012 (1067 observations), 1st August 2013 (935 observations) and 17th July 2014 (1095 observations). All years had at least 5 days with more than 200 observations. Flying ants were reported on 68 out of 97 days in both 2012 and 2013 (70%) and on 93 out of 97 days in 2014 (96%).

Spatial patterns: latitude and longitude

The mean observation day was to some extent determined by broad geographic location with significant effects of both latitude (GLMM-n; $\chi^2 = 104.53$, df = 1, $P < 0.001$) and longitude (GLMM-n; $\chi^2 = 23.90$, df = 1, $P < 0.001$) as well as their interaction (GLMM-n; $\chi^2 = 5.32$, df = 1, $P = 0.021$). Observations occurred slightly earlier in the eastern and southern parts of the country (Fig. 2). The full model, which included year as a random factor as well as latitude and longitude as fixed factors, explained 38% of the variance in

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the data (conditional $R_c^2 = 0.382$). However, only 1% of the variance was explained by the fixed factors (marginal $R_m^2 = 0.009$).

Spatial patterns: geographical clustering

The distance between observation locations across the UK on specific days was no lower than the geographical distance for comparison data points taken from across the season in any of the three study years (Table 2). This indicated an absence of significant geographical clustering. Similar results were obtained when focusing only on the Greater London region (Table 2). Despite the lack of geographical clustering at national and local (London) scales, a few *specific days* showed some geographical clustering as can be seen from the inserts in Figure 1. For example, winged ants were largely confined to the central and southern parts of the UK on 18th August 2012 (Figure 1A-IV), 16th August 2013 (Figure 1B-III) and 12th July 2014 (Figure 1C-II), while most flying ants observations occurred in northern England and Scotland on 25th August 2013 (Figure 1B-IV), Greater London on 2nd July 2014 (Figure 1C-I) and north-eastern England on 2nd September 2014 (Fig. 1C-IV). This was investigated further using the 3D bubble plot analysis described in the methods for six weather station areas widely distributed across the UK and with a large number of records.

The 3D plots (Fig. 3) showed that, even when observations were considered in 3-day blocks rather than as individual days, there was still very little synchronicity between locations in any of the survey years. In 2012, the ant flights in all six focal weather station areas were completely discrete (i.e. did not overlap with one another on the 3D plot, suggesting that ant flights were statistically different from one another in all cases). A similar pattern emerged in 2013, with most areas being discrete. Indeed, differences were quite profound, with the

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frequency plots showing that Exeter had its first major peak almost 9 days earlier than other weather stations while Glasgow had a late final peak. However, Liverpool and Nottingham (relatively close in proximity and at similar latitudes) did overlap, which suggested that ant flights at these locations were synchronized. In 2014, only Nottingham and Bristol had similar distributions. Two important conclusions can be drawn from the fact that there was little overlap between the weather station areas: (1) the lack of broad-scale synchronicity between locations even when analysed in 3-day blocks; and (2) the fact that factors driving the temporal distribution of winged ants within a given spatial area must be highly localized in origin.

Environmental correlates with mating flights: habitat and microhabitat

Habitat was found to be significant with winged ants observed in urban areas earlier than ants in rural areas (GLMM-n; $\chi^2 = 31.29$, d.f. = 1, $P < 0.001$) (Fig. 4). Overall, observations in urban areas were ca. 3 days earlier (mean day 55.5 ± 0.2, N = 7036) than in rural areas (mean day 58.5 ± 0.2, N=5286). Similarly, microhabitat was significant with ants in colonies nesting in heat-retaining (pavements, stones and brick walls) or heat-producing (compost heaps) structures being observed significantly earlier than those in other locations (GLMM-n; $\chi^2 = 94.71$, d.f. = 1, $P < 0.001$). This equated to a difference in real terms of ca. 3 days earlier (mean day 55.1 ± 0.2 (N=6543) versus 58.5 ± 0.2 (N=5779), respectively). There was also a significant interaction between nest habitat and nest microhabitat (GLMM-n; $\chi^2 = 17.04$, d.f. = 1, $P < 0.001$) (Fig. 4). However, only 1% of the variance was explained by the combination of habitat and microhabitat (marginal $R_c^2 = 0.008$),

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suggesting that although highly significant these variables only had a relatively minor effect on observation day.

Environmental correlates with mating flights: climate

Most winged ant observations occurred at temperatures above 13 °C and wind-speeds below 14 mph (6.3 m^{-1}) (Fig. 5). Indeed, the proportion of days with observations increased strongly with increased temperature (with *all* days with a mean daily temperature above 25 degrees Celsius having ants observed somewhere in the UK) and with decreasing wind speeds, while pressure did not show any clear pattern (Fig. 5).

Observations were significantly positively influenced by temperature (GLMM-b; χ^2 = 297.2, d.f. = 1, $P < 0.001$), negatively influenced by wind speed (GLMM-b; $\chi^2 = 63.4$, d.f. = 1, $P < 0.001$), and were also influenced by positive temperature trends (i.e. temperature increase relative to the previous day: GLMM-b; $\chi^2 = 132.1$, d.f. = 1, $P < 0.001$) and negative wind speed trends (i.e. wind speed decrease relative to the previous day: GLMM-b; $\chi^2 = 33.2$, d.f. = 1, $P < 0.001$). However, neither pressure (GLMM-b; $\chi^2 = 0.2$, d.f. = 1, $P = 0.687$) nor the difference in pressure from the previous day (GLMM-b; $\chi^2 = 1.3$, d.f. = 1, $P = 0.249$) were significant, although the interaction between temperature and pressure was significant (GLMM-b; $\chi^2 = 60.3$, d.f. = 1, $P < 0.001$). Winged ant observations occurred within a pressure range of 1004 mbar and 1028 mbar. All other interactions were not significant. The final model explained 51% of the variance in the data (conditional $R_c^2 = 0.514$), with 44% of the variance explained by the fixed effects alone (marginal $R_m^2 = 0.442$).

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Discussion

Using citizen science allowed us to gather the largest dataset on mating flights of any ant species anywhere in the world. Because participants were asked to record relatively simple information, we have confidence that the data we collected were reliable despite the fact that individual data points could not be validated (a common criticism of citizen science: Follett and Strezov 2015). Moreover, the enhanced participation in 2013 enabled us to identify the species of ants being reported .The fact that 88.5% of the ants we identified were *Lasius niger* means that although it was not possible to assign species to any given record, we can be reasonably confident that our findings can be applied to this species.

Temporal patterns

In terms of temporal patterns in *Lasius* mating flights, July and August were the peak months in all three years (coinciding with the peak of the UK summer) accounting for 97% of records. A potential issue with using citizens to record data is that it is rarely possible to quantify effort, and patterns of observations collected in time (or space) can simply reflect the distribution of citizens rather than the focal phenomenon (e.g. Dickinson et al. 2010). This cannot be discounted in this study, since the peak recording months coincide with the summer holiday period. However, the extreme relative density of observations during July and August indicates that this is a reflection of ant rather than human behaviour; it is unrealistic to assume that people are more than 40 times more likely to be outside in July and August than in June and September. Also, in this study we found that the peak observation day was Wednesday (Figure 1), indicating that people were not more likely to record observations at weekends. There were considerable differences in temporal pattern

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between years. In particular, both the number and relative timing of the peaks differs, as does the duration of the flying season (over one third longer in 2014 compared with 2012 and 2013). There is, therefore, broad synchrony in terms of the general time of year, but a lack of any predictable temporal patterns within that time. The inference of this is that ant reproductive flights occur in response to triggers that are not themselves fixed in time, for example, seasonally-variable environmental or climatic factors rather than, say, day length or absolute date. This is a pattern found in other, more limited, studies of ant mating flights (e.g. do Nascimento et al. 2011).

Spatial patterns

The UK is aligned such that its two longest perpendicular axes are approximately north-south and east-west. This results in a pronounced progression of some temporal phenomena, such as the first signs of spring, with the "front" moving northwards as the season progresses. We found a similar broad northwards, and also westwards, progression of records over time. However, despite being significant statistically, the effect of latitude and longitude were small. This reinforces the inference that local conditions are more important to the timing of mating flights than overall geographical location. The importance of local conditions was further highlighted by our analyses of spatial clusters, which revealed no significant clustering of observations across the UK when considered at a national level. This absence of spatial clustering was also apparent in Greater London. In other words, observations of winged ants could occur in any given area throughout the observation period and even examining observations at a much finer spatial scale for a smaller geographical region did not increase the spatial coordination.

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Despite there being no overall significant clustering of observations (and therefore, presumably, mating flights) at a national level, on a few specific days observations were largely confined to geographically discrete regions (e.g. central and southern UK on 18th August 2012). A more detailed analysis of the data for six geographically-separated areas (Glasgow, Liverpool, Nottingham, Bristol, London and Exeter) taken in 3-day blocks confirmed both the absence of spatial coordination at a national level and the broad longitudinal trend discussed above, with Exeter (in the far south of UK) having observations 9 days earlier than more northerly weather stations. It also showed that, for each of the three years, the focal locations generally had temporal patterns that were statistically discrete from one another, suggesting that even when observations were examined at a very fine temporal scale, spatial co-occurrence was not increased.

Environmental correlates

Previous studies of ant mating flights have indicated the likely importance of weather in determining the timing of flights (e.g. Boomsma and Leusink (1981); Depa (2006); do Nascimento et al. 2011; Gomez and Abril 2012; Staab & Kleineidam, 2014). The UK has highly variable weather both through time and space and if local weather conditions were important then it would make sense that mating flights would only tend to demonstrate strong synchrony and coordination at a local level. The results of our meteorological data analysis showed that temperature and wind speed were significant factors; observations only occurred above 13°C and at wind speeds below 14mph (6.3 ms^{-1}). Recent precipitation has been shown to be important in the leafcutting ant *Atta vollenweider* (Staab & Kleineidam, 2014) but because only monthly totals were available we were unable to include this factor in our study. However, *Atta* live in neo-tropical conditions with

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pronounced dry-wet season cycles and hard clay soils that cannot be dug before the wet season begins (Staab and Kleineidam, 2014), whereas the UK has a maritime, temperate climate with frequent and abundant rainfall throughout the year.

Previous studies have supported the concept of a threshold temperature, above which further increases do not necessarily result in increased flight activity (e.g. as demonstrated by trapping records related to temperature by Taylor (1963)). However, we found a significant linear increase in observations as temperature increased above the threshold of 13°C. This finding could be an artefact of the method of data collection since people may be more likely to be outside when it is warmer. However, we favour an alternative, biological explanation: insects fly more efficiently and powerfully at higher temperatures (e.g. Stevenson and Josephson 1990), which reduces the time spent in the air searching for a mate and searching for a nest site on the ground and therefore reduces predation risk. Avoiding high winds (also Boomsma and Leusink (1981)) makes biological sense since mating in-flight would be difficult or impossible at higher wind speeds. The ability of ants to detect temperature changes is well known (e.g. Ruchty et al. 2010) but it is intriguing that mating flights were more likely to occur when temperature was rising and wind speed failing relative to the day before, suggesting that ants are able to evaluate change in local conditions over time.

Local conditions with respect to habitat and microhabitat also proved to be significant in the timing of observations although, as with latitude and longitude, the effect was weak. Colonies in urban areas (which are often associated with elevated temperatures; the urban heat island effect (Bornstein 1968)) and those associated with heat retaining structures had observations, on average, 3 days earlier. Taken together, the most likely explanation for this

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is that such colonies develop in slightly warmer environments and so have winged ants ready to fly at an earlier date (see below). Potentially, this could allow increased productivity and the possibility of producing multiple batches of reproductives in such colonies. However, other factors including perceived elevated air temperatures and reduced wind in urban and sheltered habitats/microhabitats could also play a role in promoting earlier flights.

Conclusions

Local weather conditions, geographical location, habitat and microhabitat are extrinsic factors that interact with intrinsic factors to influence the precise timing of mating flights in *Lasius niger*. Colonies overwinter in an inactive state and begin to be active during spring so the timing of spring, the ability of colonies to find food as well as the temperature and other weather factors will combine to determine precisely when individual colonies can produce reproductives. Many colonies can be "flight ready" by July (or earlier, since some flights were recorded here in June), but variation caused by colony-specific differences and geographical location may mean that some colonies are not ready to launch mating flights until August. Nests may also produce multiple batches of reproductives in favourable seasons and have multiple flights (an initial major flight and subsequent minor flights) (Hölldobler and Wilson 1990). Overall, we conclude that the timing of *Lasius niger* flights is determined by a combination of factors that include: the broad scale geographical location within the country (north and west tend to fly later but the effect was weak); the habitat and microhabitat of the colony (urban colonies and those associated with heat retaining structures tend to fly earlier but again the effect was weak); the current temperature and wind speed in relation to thresholds we estimate to be 13°C and 6.3 ms^{-1}

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respectively (a strong effect); and the favourable or detrimental changes in these weather factors from the previous day.

Ant mating flights are charismatic mass phenomena that have been difficult to quantify at large spatial scale across multiple flying seasons. For the first time, we were able to collect thousands of data points at a national scale using a robust citizen science approach. Our data confirm that the timing of *Lasius niger* flights is likely based on highly conserved weather-based thresholds that can be assessed relatively precisely at a colony-level by colonies able to assess both current weather conditions and recent changes.

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References

Bates, D. et al. 2015. Fitting Linear Mixed-Effects Models Using lme4. – J. Stat. Softw. 67: 1-48.

Berger, J. 2004. The last mile: how to sustain long-distance migration in mammals. – Conserv. Biol. 18: 320-331.

Boomsma, J.J. and Leusink, A. 1981. Weather conditions during nuptial flights of four European ant species. – Oecologia 50: 236-241.

Boone, R.B. et al. 2006. Serengeti wildebeest migratory patterns modeled from rainfall and new vegetation growth. – Ecology 87: 1987-1994

Bornstein, R.D. 1968. Observations of the urban heat island effect in New York City. – J. Appl. Meteorol. 7: 575-582.

Catlin-Groves, C.L. et al. 2009. Use of confidence radii to visualise significant differences in principal components analysis: Application to mammal assemblages at locations with different disturbance levels. – Ecol. Inform. 4: 147-151.

Corbet, P.S. 1958. Lunar periodicity of aquatic insects in Lake Victoria. – Nature 182: 330-331.

'This article is protected by copyright. All rights reserved.'

Accepted Article

Depa, L. 2006. Weather conditions during nuptial flight of *Manica rubida* (LATREILLE, 1802 (Hymenoptera: Formicidae) in southern Poland. – Myrmecol. Nachr. 9: 27-32.

Dickinson, J.L. et al. 2010. Citizen science as an ecological research tool: challenges and benefits. – Annu. Rev. Ecol. Evol. Syst. 41: 149-72.

do Nascimento, I.C. et al. 2011. Phenology of mating flight in Ecitoninae (Hymenoptera: Formicidae) in a Brazilian Atlantic Forest location. – Ann. Soc. Entomol. Fr. 47: 112-118.

Feitosa, R.M. et al. 2016. Diurnal flight periodicity of a Neotropical ant assemblage (Hymenoptera, Formicidae) in the Atlantic Forest. – Rev. Bras. Entomol. 60: 241-247.

Field, A.P. 2000. Discovering Statistics using SPSS for Windows. – Sage Publications Ltd

Follett, R. and Strezov, V. 2015. An analysis of citizen science based research: usage and publication patterns. – PloS ONE 10: e0143687.

Fox, J. and Weisberg, S. 2011. An {R} Companion to Applied Regression Second Edition. – Sage, USA.

Gelman, A. and Su, Y.S. 2014. Arm: Data Analysis Using Regression and Multilevel/Hierarchical Models. – R Package Version 1.7-07.

'This article is protected by copyright. All rights reserved.'

Accepted Article

Glass, G.V. et al. 1972. Consequences of failure to meet assumptions underlying the fixed effects analyses of variance and covariance. – Rev. Educ. Res. 42: 237-288.

Goodenough, A.E. et al. 2012. Regression with empirical variable selection: description of a new method and application to ecological datasets. – PLoS One 7: e34338.

Gomez, C. and Abril, S. 2012. Nuptial flights of the seed-harvester ant *Messor barbarus* (Linnaeus, 1767)(Hymenoptera: Formicidae) in the Iberian Peninsula: synchrony, spatial scale and weather conditions. – Myrmecol. News 16: 25-29.

Hart, A. et al. 2012 The role of citizen science and volunteer data collection in zoological research. – Int. J. Zool. Article ID: 105345.

Howard, E. and Davis, A.K. 2009. The fall migration flyways of monarch butterflies in eastern North America revealed by citizen scientists. – J. Insect Conserv. 13: 279-286.

Huber, M.P. 1820. The Natural History of Ants, First Edition. – Longman, Hurst, Rees, Orme and Brown.

Hölldobler, B. and Wilson, E.O. 1990. The Ants. – Harvard University Press.

Kaspari, M. et al. 2001. The reproductive flight phenology of a neotropical ant assemblage. – Ecol. Entomol. 26: 245-257.

'This article is protected by copyright. All rights reserved.'

Accepted Article

Myers, R. (1990) Classical and Modern Regression with Applications (2nd edn). – Boston.

Nakagawa, S. and Schielzeth, H. 2013. A general and simple method for obtaining R^2 from generalized linear mixed-effects models. – Methods Ecol. Evol. 4: 133-142.

Rubenstein, D.I. 1978. On predation, competition, and the advantages of group living. – In: Bateson P.P.G. and P.H. Klopfer (eds). Perspectives in Ethology Volume 3. Plenum Press, pp 205-231.

Ruchty M. et al. 2010. Detection of minute temperature transients by thermosensitive neurons in ants. – J. Neurophysiol. 104: 1249-1256.

Simon, C. 1988. Evolution of 13-and 17-year periodical cicadas (Homoptera: Cicadidae: Magicicada). – Bull. Entomol. Soc. Am. 34: 163-176.

Skinner, G.J. and Allen, G.W. 1996. Ants. Naturalists' Handbook 24. – Richmond Publishing Company Limited.

Smith, D.R. 2002. Spatial and temporal distribution of horseshoe crab (*Limulus polyphemus*) spawning in Delaware Bay: implications for monitoring. – Estuaries 25: 115-125.

'This article is protected by copyright. All rights reserved.'

Accepted Article

Staab, M. and Kleineidam, C.J. 2014 Initiation of swarming behavior and synchronization of mating flights in the leaf-cutting ant *Atta vollenweideri* Forel, 1893 (Hymenoptera: Formicidae). – Myrmecol. News 19: 93-102.

Stafford, R. 2012. Inferential and visual analysis of ethogram data using multivariate techniques. – Anim. Behav. 83: 563-569.

Stevenson, R.D. and Josephson, R.K. 1990. Effects of operating frequency and temperature on mechanical power output from moth flight muscle. – J. Exp. Biol. 149: 61-78.

Taylor, L.R. 1963. Analysis of the effect of temperature on insects in flight. –J. Anim. Ecol. 32: 99-117.

Thomas, R. et al. 2013. Data Analysis with R statistical Software: A Guidebook for Scientists. – Eco-Explore, Newport.

Thornhill, T. 2010. Flying ants plague London. – Metro 19th July 2010 http://metro.co.uk/2010/07/19/flying-ants-plague-london-451833/.

Walls, E.A. et al. 2002. The horseshoe crab, *Limulus polyphemus*: 200 million years of existence, 100 years of study. – Rev. Fisheries Sci. 10: 39-73.

'This article is protected by copyright. All rights reserved.'

Accepted Article

Wilson, E.O. 1957. The organization of a nuptial flight of the ant *Pheidole sitarches* Wheeler. – Psyche 64: 46-50.

Wood, C. et al. 2011. eBird: engaging birders in science and conservation. – PLoS Biol. 9: e1001220.

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Table Legends

Table 1: Locations of the six weather stations used to analyze mid-scale geographical clustering

Met Office ID	Location (nearest city)	Lat.	Long.	Elevation (m)	N
3134	Bishopton (Glasgow)	55.91	-04.53	59	237
3316	Crosby (Liverpool)	53.49	-03.04	11	491
3354	Watnall (Nottingham)	53.01	-01.25	117	633
3628	Filton (Bristol)	51.51	-02.58	87	608
3781	Kenley (London)	51.30	-00.09	170	800
3839	Exeter Airport (Exeter)	50.74	-03.41	27	247

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Table 2: Distance between actual flights on specific days (D_{actual}) and comparison data points from across the season (D_{mean}) at national and regional scales with significance determined using sign tests (see Methods). Note that the number of days (N_{total}) differs between years because distances between points could only be calculated for days with ≥2 observations.

	Distances		Sign tests		
	D_{actual} ± SEM	D_{mean}	N_{nearer}	N_{total}	P
National 2012	2.52 ± 0.14	2.31	31	60	0.90
National 2013	2.31 ± 0.08	2.44	34	59	0.30
National 2014	2.24 ± 0.09	2.38	51	84	0.07
London 2012	0.23 ± 0.02	0.27	20	29	0.06
London 2013	0.25 ± 0.01	0.26	17	24	0.06
London 2014	0.21 ± 0.02	0.23	17	28	0.34

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