

Reproductive phenologies in a diverse temperate ant fauna

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Abstract. 1. Ant nuptial flights are central to understanding ant life history and ecology but have been little studied. This study examined the timing of nuptial flights, the synchronicity of nuptial flights (as a potential index of mating strategy), and variation in nuptial flights with elevation and among years in a diverse temperate ant fauna.

2. Flights occurred throughout the year, but were concentrated in the beginning of summer and in early fall (autumn). Relative to the entire flight season, closely related species tended to be more likely than expected by chance to fly at similar times, perhaps because of phylogenetic constraints on life history evolution.

3. Flights were relatively synchronous within species for nearly all species considered, but synchronicity did not appear to be a robust estimate of overall mating strategy.

4. Overall patterns in nuptial flights among species and the timing of flights for individual species varied with elevation, but did not vary greatly among years.

5. Although this study is one of the most comprehensive on the reproductive flight phenologies of ants, much remains to be learned about the causes and consequences of such spatial and temporal variation in flight phenology.

Key words. Ant diversity, Formicidae, life history evolution, mating, reproduction.

Introduction

Ants are abundant and dominant members of almost every terrestrial ecosystem (Hölldobler & Wilson, 1990). Yet surprisingly little is known about their reproductive ecology, especially in temperate forests where ants can act as key ecosystem modifiers, dispersal agents, predators, and mutualists (e.g. Folgarait, 1998). One component of a taxon's reproductive ecology, the timing of reproduction, influences the timing of subsequent life stages, the potential for hybridisation, the forms of reproduction that are possible, and even potentially the ability of species to coexist in local assemblages (e.g. Kaspari *et al.*, 2001a). Although reproductive phenologies have been well studied in plants (e.g. Price & Waser, 1998) and birds (e.g. Tryjanowski *et al.*, 2005), few studies of annual reproductive phenologies of entire ant assemblages exist, and the most comprehensive of those studies are from the tropics or subtropics (Tschinkel, 1991; Kaspari *et al.*, 2001a,b; Torres *et al.*, 2001).

Key to beginning to understand patterns and constraints on the timing and mode of reproduction in ants is an understanding of the distribution of ant reproductive flights across space, time and species (e.g. Tschinkel, 1991). In this paper, patterns of reproductive flights for a diverse ant assemblage in Great Smoky Mountains National Park (GSMNP, U.S.A.) are considered, and three related questions are addressed. (1) Are reproductive flights of congeneric species more or less staggered with respect to one another than expected by chance? (2) Are the flights of individual species synchronous or asynchronous in time and space? (3) How does flight phenology vary along an environmental gradient and between years?

Are reproductive flights of congeneric species staggered?

Biologists have long speculated that closely related species might use behavioral mechanisms to avoid hybridisation (e.g. Stiles, 1975) and its associated costs. In ants, the staggering of flight phenologies of closely related species would be one way that closely related ants might avoid hybridisation. Several studies have anecdotally shown that species within genera are staggered

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within a particular day (e.g. day flyers versus night fliers; Kannoški, 1959; Haddow *et al.*, 1966; Hölldobler, 1976), but staggering across months may provide a more complete barrier to hybridisation, as the probability of overlap among mating flights is much lower. By this argument, flight times of two closely related species should be more different or more staggered than the flight times of any two species drawn at random from the assemblage. Alternatively, ant flight times may be phylogenetically constrained (e.g. Hölldobler & Wilson, 1990; Kaspari *et al.*, 2001a), such that closely related ants might be more likely to fly at the same time. Neither of these possibilities can be precluded on the basis of existing knowledge about temperate ant assemblages. In the ant fauna of GSMNP, whether flights of congeners are more staggered in time than expected by chance is considered.

Are flights of species synchronous or asynchronous?

In addition to when to initiate flights, ant species may vary in the duration of their mating flights. Flight events may be as short as a particular afternoon on a single day (Talbot, 1965; Hölldobler & Wilson, 1990) or so long that they are essentially continuous throughout the year (e.g. Kaspari *et al.*, 2001a). In ants, the duration of the mating flights may be linked to particular mating behaviors. On the basis of published records, two types of mating behaviors seem to predominate in ants (Hölldobler & Bartz, 1985; Hölldobler & Wilson, 1990). The most obvious mating flights are those of species that engage in lek mating in which males either fly in large swarms or gather in central places where females choose among males. Because lek mating depends on large male aggregations, lek matings tend to be short in time and are assumed to be synchronous across large areas.

Less obvious, but perhaps more globally common (e.g. Hölldobler & Bartz, 1985; Kaspari *et al.*, 2001a), are species in which females call to males. In female calling mating systems, flights tend to be asynchronous and may last many months with occasional males flying out to search for calling females. Hölldobler and Wilson (1990) and later Kaspari *et al.* (2001a) suggest that highly synchronous flights are a potential signal of lek mating, whereas asynchronous flights can be interpreted as evidence of female calling systems. Because females do not need to fly in female calling mating systems, it can also be expected that, in such systems, most individual ants seen or caught flying will be males. The predominance of these two systems in ants or how they vary with population size (because as population size decreases, the need for synchronous flight might increase) remains unknown. Here, the synchrony and indirectly the mating behavior of the flights of common ants in the GSMNP across years and elevations are examined.

Do reproductive flights vary with environmental variation and among years?

Because the signals thought to be responsible for triggering ant mating flights, such as extreme rainfall and temperature events, are likely to vary across space, it can be expected that mating flights of spatially separated populations might occur at different times as a

consequence of different timing of the triggers for flights. In some bird species, neighboring populations have been shown to mate at different times of the year because of the local timing of mating cues and as a consequence have reduced gene flow between them (Moore *et al.*, 2005). A similar pattern may occur in the ant assemblages examined here. For example, high elevation and low elevation populations may experience the first rain of the season on different days, and as a consequence those species that fly immediately after the first rain may fly on different days or even months at high and low elevations. In the southern Appalachians, the intensity and frequency of precipitation events increase with elevation. In addition, differences among years in the order or magnitude of mating cues may lead to differences in the flight times of species among years. Thus it can be predicted that high elevation and low elevation populations may differ in any given year in the timing of their peak mating flights, and flight times may vary among years.

Methods

Data in this study come from captures of alates from paired Malaise traps at 11 sites over 3 years in GSMNP, U.S.A. as part of the All Taxa Biodiversity Inventory (<http://www.dLia.org/atbi/>). The GSMNP ranges in elevation from 256 m to 2025 m. Approximately 79% of the gradient in elevation within the park was sampled and included the highest forested area within the park (Clingman's Dome). A single HOBO datalogger was installed at each site and monitored for the entire year to record temperature at each site. At each site, the Malaise traps were placed on the ground 75–100 m from one another. The contents of the traps were collected every 2 weeks from January 1999 through January 2002 for a total of ≈ 24 390 trap days (two traps per site \times 11 sites \times 365 days \times 3 years), which compares favorably with the Malaise trapping for other work in the tropics (1456 trap days; Kaspari *et al.*, 2001a,b). Voucher specimens are in our collections at the University of Tennessee and North Carolina State University and at GSMNP.

Ant alates were associated with workers and hence identified with species by matching alates to colony collections made by us in GSMNP, from keys for genera in Smith (1943), and through comparison with digital images of type specimens in the California Academy of Sciences Collection (www.antweb.org). In the few cases where individuals collected represented morphologically distinct species but could not be tied to known species, those individuals were identified as morphospecies. Species-level analyses are focused on queens, except where males were easily distinguished [e.g. *Prenolepis imparis* (Say 1836), *Myrmecina americana* (Emery 1895)], and males are considered primarily at the genus and subfamily levels. Monthly time intervals, based on a calendar of 4-week lunar months, were the focus of this study, even though ants were collected biweekly, because the exact sampling date varied among years and to aid comparison with previous studies (as in Kaspari *et al.*, 2001a).

Are reproductive flights of congeneric species staggered?

To test whether flight times of congeners are more or less likely than random to be staggered, the observed flight times

of congeneric species were compared, with the null expectation that flight times within each genus are random throughout the activity period (first to last flight date for all species combined). Only genera with three or more species were considered. Analyses were performed using co-occurrence analyses in the program EcoSim 7.0 (Gotelli & Entsminger, 2005). In the analyses, the number of months each species flew was held constant, and the identity of the months in which each species flew was randomised. The percentage of a species' total activity in a given month rather than raw abundance data, following Kaspari *et al.* (2001a), was used for this test to control for differences among species in the number of individuals captured. To replicate the methods of Kaspari *et al.* (2001a), only species within a genus were randomised only among those months in which that genus was collected. However, because this may artificially restrict the possible months in which a species could be active, a second set of randomisations was conducted. In this set, species within each genus considered were randomised across the entire year. The second set of randomisations best tests the question, "have species within a genus evolved flight periods at random within the year or are they clustered relative to one another?" The output of the randomisation analyses of flight timing is a variance ratio test index, which indicates the degree to which flights are either clumped or staggered in time for a given taxon; the larger the variance is, the less clumped the flights for species within that genus are.

In addition to the co-occurrence analyses, ANOVAS were used to test the hypothesis that variation in peak flight occurrence among species was explained by their generic identity. That is, just as in the randomisation tests of flight timing, do closely related species tend to have similar flight times? Separate ANOVAS were conducted with genus and subfamily as factors. Analyses were performed for all collected species and also for just the subset of those species used in the variance tests.

Are flights of species synchronous or asynchronous?

The synchrony of the reproductive flights of ant species represented by more than 10 individuals are also examined. Synchrony was measured as in Kaspari *et al.* (2001a) as the coefficient of variation of reproductive activity among months (standard deviation in number of ants in a month/mean number of ants in a month \times 100), where higher values indicate greater synchrony of flights. To test whether differences in synchrony among species are associated with their lineage, two separate ANOVAS were used, with genus or subfamily as the independent categorical variable. The expectation is that, if synchrony is evolutionarily conserved, then closely related species will have similar levels of synchrony, and hence the variation among genera (or subfamilies) will be greater than the variation within.

Do reproductive flights vary with environmental variation and among years?

To examine the relationship between elevation and flight phenology, the overall patterns of alate flights at three low elevation

sites (below 1000 m) were compared with the patterns at eight high elevation sites (above 1000 m). In addition, for those species that were captured at both low elevation and high elevation sites, the peak in reproductive flights at low and high elevation were compared. The frequency with which low elevation species flew earlier, later, and at the same time as high elevation species were evaluated using a χ^2 test.

To test whether peak flight times within a species were concordant both among years and between high elevation and low elevation sites within GSMNP, Kendall's analysis of concordance (Zar, 1999) was used. The test statistic, W , may range from 0 (when there is no concordance among peak flight time among years or between high elevation and low elevation sites) to 1 (when there is complete concordance, i.e. when peak flight times occur at exactly the same time).

Results

Overall patterns

In total, 57 ant species and 13 226 individual alates were collected. The number of species collected represents roughly 70% of all species known from the entire park (e.g. Cole, 1940; Dunn *et al.*, 2006). Species of the litter ant genus *Pyramica* were poorly represented (one species out of no fewer than six possible, N. J. Sanders, unpublished), but most other genera were well accounted for in the sampling. Alate catch varied from just one individual alate in late December (lunar month 13) to 6583 total collected alates in the 9th lunar month during the late summer/early fall (autumn). Individual species varied in when they reproduced. A relatively small number of species (*Camponotus* spp., *Prenolepis imparis* (Say 1836)) flew in the early spring (Fig. 1a). Several species flew in the mid to late summer (Fig. 1b), including several *Myrmica* species. Finally, many species, including *Amblyopone pallipes* (Haldeman 1844), *Myrmica punctiventris* (Roger 1863), *Myrmecina americana* (Emery 1895), and *Aphaenogaster rudis* (Enzmann 1947), flew at the end of the fall (Fig. 1c).

Are flights of congeners staggered?

Congeneric ant species in GSMNP tend to fly at similar times. On the basis of the variance ratio test, congeners did not appear to be more likely to have staggered flights than expected if their reproductive flight phenologies were arranged at random. In fact, in five of the seven genera examined, congeners flew closer in time to one another than expected by chance when the entire year was considered. Species within genera tended to be randomly distributed with respect to each other when only the months in which a genus was recorded flying were considered (Table 1).

Generic identity explained 46% of the variation in peak flight date when all species were considered ($R^2 = 0.46$, $F_{36,57} = 2.97$, $P = 0.002$). When only those genera that were used in the variance ratio test were considered, a similar amount of the variation in peak flight date was explained (adjusted $R^2 = 0.43$,

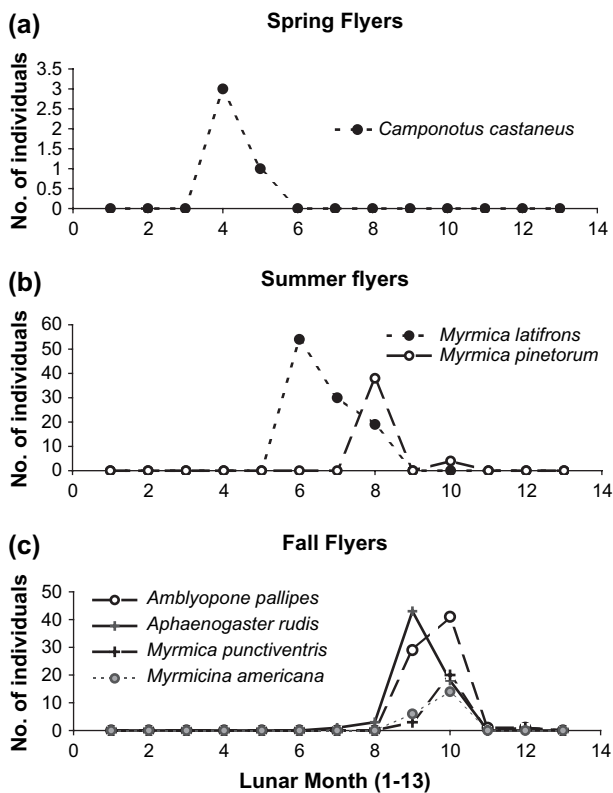


Fig. 1. Reproductive flights of representative species flying in the spring (a), summer (b) and fall (c).

$F_{36,45} = 12.3$, $P < 0.0001$). The model including subfamily was not significant for all species ($R^2 = 0.081$, $F_{53,57} = 2.65$, $n = 57$, d.f. = 3, $P = 0.058$), but was significant when just those genera used in the variance ratio test were considered ($R^2 = 0.12$, $F_{41,45} = 22.6$, $n = 45$, d.f. = 3, $P = 0.03$).

Are flights of species synchronous or asynchronous?

Relative to the timing of reproductive flights in tropical ant assemblages (Kaspari *et al.*, 2001a,b), the timing of reproductive flights within species in the assemblages in GSMNP are synchronous (Table 2). However, this is not to say that all species showed single-day mass eruptions. In fact, in any given year most species flew in more than one month, with the median number of months in which queens of a particular species flew at 1.7 months. As might be predicted, when flight records were pooled among years, species tended to appear less synchronous than when each year was considered separately.

The length of flight seasons did not vary significantly among subfamilies (adjusted $R^2 = 0.031$, $F_{53,57} = 0.44$, $P = 0.73$) or genera (adjusted $R^2 = 0.13$, $F_{36,57} = 0.241$, $P = 0.99$). Season length did not correlate with the number of individuals collected among species (adjusted $R^2 = 0.012$, $F_{1,57} = 1.7$, $P = 0.19$).

Species with female calling mating systems are expected to be better represented by males in the collections than are species with lek mating systems. Overall, most alates collected in traps were males regardless of the ant subfamily considered (Table 1). In all subfamilies except the Dolichoderinae (represented by just two species), males made up more than 70% and often more than 90% of individuals. The proportion of individuals collected that was male was similar to the results of Kaspari *et al.* (2001b), but much higher than the sex ratios reported from colony level

Table 1. Patterns in flight timing for seven genera with three or more species recorded in this study. No. of months indicates the number of months over which the randomisations were conducted. No. species indicates number of species of the genus included in the analyses. Variance index is the observed variance ratio. Mean sim. is the simulated variance ratio. When the observed variance ratio is above the simulated ratio it can be interpreted as evidence of clustering of species in time; when it is less than the simulated ratio, the pattern can be interpreted as staggering in time. *Significant deviation from random.

Genus	No. of months	No. of Species	Variance index	Mean Sim	$P <$	$P >$
Randomisations across all months						
<i>Aphaenogaster</i>	13	4	1.42	1.00	0.93	0.19
<i>Camponotus</i>	13	6	2.9	0.99	0.99	0.001*
<i>Crematogaster</i>	13	4	1.92	0.99	0.99	0.03*
<i>Formica</i>	13	6	2.16	0.99	0.99	0.01*
<i>Lasius</i> (including <i>Acanthomyops</i>)	13	7	0.87	0.99	0.5	0.71
<i>Myrmica</i>	13	6	0.98	0.99	0.64	0.58
<i>Stenamma</i>	13	5	2.4	1.01	0.99	0.001*
<i>Temnothorax</i>	13	3	1.35	1.00	0.98	0.34
Randomisations across active months						
<i>Aphaenogaster</i>	4	4	0.33	0.99	0.99	0.11
<i>Camponotus</i>	5	6	2.1	1.00	0.95	0.08
<i>Crematogaster</i>	4	4	1.3	0.99	0.87	0.44
<i>Formica</i>	7	6	1.3	1.00	0.79	0.32
<i>Lasius</i> (including <i>Acanthomyops</i>)	12	7	0.76	1.00	0.38	0.82
<i>Myrmica</i>	8	6	0.22	1.00	0.053	1.00
<i>Stenamma</i>	4	5	0.65	0.99	0.56	0.77
<i>Temnothorax</i>	3	3	0.33	1.00	0.56	1.00

Table 2. Synchrony within ant species for which more than 10 alate queens were collected. Results are for averages among years and overall with records of flights summed across years (as in Kaspari *et al.*, 2001a). Peak month indicates the mean month in which the most individuals were collected across years. No. of individuals indicates the total number of individuals collected across years. Standard error in parentheses. Species of *Stenamma* could not be consistently allied to workers and are thus identified only with morphospecies.

Genus species	CV by year (SE)	CV (overall)	No. of years	Peak month (SE)	No. of individuals
<i>Acanthomyops claviger</i> (Roger 1862)	304.9 (96.2)	162.27	3	7 (5.6)	12
<i>Amblyopone pallipes</i> (Haldeman 1844)	196.3 (21.7)	255.12	3	9.5 (0.6)	48
<i>Aphaenogaster rudis</i> (Enzmann 1947)	302.1 (46.7)	212.63	3	8.9 (0.7)	14
<i>Brachymyrmex depilis</i> (Emery 1893)	314.9 (64.5)	296.82	1	10.5 (0.7)	11
<i>Crematogaster pilosa</i> (Emery 1895)	319.2 (58.5)	228.88	2	9 (0)	15
<i>Hypoponera opacior</i> (Forel 1893)	268.7 (12.9)	183.06	2	7.5 (2.1)	109
<i>Lasius alienus</i> (Foerster 1850)	291.2 (72.9)	295.16	3	7.8 (0.6)	17
<i>Myrmica pinetorum</i> (Wheeler 1905)	321.7 (67.7)	198.02	3	8.5 (2.2)	141
<i>Myrmica latifrons</i> (Starcke 1927)	295.2 (39.9)	295.18	2	7 (1.4)	29
<i>Myrmica punctiventris</i> (Roger 1863)	292.7 (93.7)	252.14	3	9.8 (0.6)	21
<i>Myrmecina americana</i> (Emery 1895)	317.9 (73.9)	244.50	3	10 (0)	19
<i>Ponera pennsylvanica</i> (Buckley 1866)	270.0 (82.3)	273.99	3	9.3 (0.6)	21
<i>Stenamma spgh</i> (morphospecies)	331.1 (41.7)	254.13	2	9.5 (0.7)	26
<i>Stenamma spsm</i> (morphospecies)	313.1 (67.2)	199.67	2	10 (4.2)	10
<i>Tapinoma sessile</i> (Say 1836)	235.1 (12.7)	249.00	2	7.5 (0.7)	72
<i>Temnothorax longispinosus</i> (Roger 1863)	308.6 (60.8)	244.10	3	8.25 (0.5)	12
Overall (average) GSMNP	292.7	240.3			

studies of ants in the region (e.g. Nonacs, 1986). However, the results for subfamilies are biased for Formicinae, Myrmecinae and Ponerinae by a few genera with disproportionately high numbers of males, *Ponera* + *Hypoponera* in the Ponerinae (males of the two genera could not reliably be distinguished), *Prenolepis imparis* (Say 1836) in the Formicinae and *Myrmecina americana* (Emery 1895) the Myrmecinae (Table 3).

Do reproductive flights vary with elevation and among years?

Temporal patterns in both the number of species and the total number of individuals captured by the Malaise traps depended on elevation (Figs 2 and 3). In low elevation sites, reproductive flights occurred early in the summer and in some sites again in the late fall. In contrast, in high elevation sites, there was a single pulse of reproductive flights in lunar months 8–10 (Figs 2 and 3). The difference between low elevation and high elevation sites was not due to differences between low and high elevations in terms of the flight times of individual species. Twenty species occurred at both high elevation and low elevation sites. Of these species, 10 had earlier flights at low elevations and three had earlier flights at high elevations, and seven were the same across elevations in terms of peak flight time ($\chi^2 = 2.34$, $n = 20$, d.f. = 2, $P = 0.31$). Instead, the difference between low and high elevations was largely due to the presence of several abundant, early flying ant species at low elevations that were absent higher up (Fig. 1). In particular, *Camponotus* species, *Acanthomyops claviger* (Roger 1862) and *Prenolepis imparis* (Say 1836) flew early and were abundant in traps in low elevation sites, but were absent from higher elevation sites.

The peak flight times of species were generally concordant among years ($W_{11,12} = 0.62$, $P < 0.05$), indicating that species tended to fly in roughly the same order each year. There was, however, little evidence that high and low elevation populations

of the same species tended to fly at the same time in the same year ($W_{20,21} = 0.50$, $P < 0.05$), although as the χ^2 tests indicate, the direction of discordance among high and low elevation populations was not consistent among species.

Table 3. Reproductive output by sex and subfamily. Although Ecitoninae (*Neivamyrmex* spp.) have been recorded by us within the park, no individuals were recorded in Malaise traps. In Kaspari *et al.* (2001a) male dolichoderines and formicines were not separated, so a combined ratio for the two groups is presented here.

Subfamily	Males	Alate females	Sex ratio (GSMNP)	Sex ratio (BCI)
Dolichoderinae + Formicinae	6446	290	0.96	0.92
<i>Acanthomyops</i>	8	17	0.29	
<i>Brachymyrmex</i>	3	14	0.16	
<i>Camponotus</i>	43	20	0.68	
<i>Formica</i>	9	38	0.19	
<i>Lasius</i>	140	118	0.54	
<i>Paratrechina</i>	6	5	0.54	
<i>Prenolepis</i>	6229	2	0.99	
<i>Tapinoma</i>	9	65	0.12	
Myrmecinae	1201	421	0.74	0.97
<i>Aphaenogaster</i>	43	66	0.40	
<i>Crematogaster</i>	27	24	0.53	
<i>Myrmica</i>	25	209	0.10	
<i>Myrmecina</i>	849	23	0.99	
<i>Pyramica</i>	13	1	0.92	
<i>Stenamma</i>	164	77	0.67	
<i>Temnothorax</i>	1113	16	0.99	
Ponerinae	6851	111	0.98	0.98
<i>Amblyopone</i>	217	72	0.75	
<i>Ponera</i> + <i>Hypoponera</i>	5567	37	0.99	
<i>Proceratium</i>	33	2	0.94	
Total	14498	822	0.94	0.98

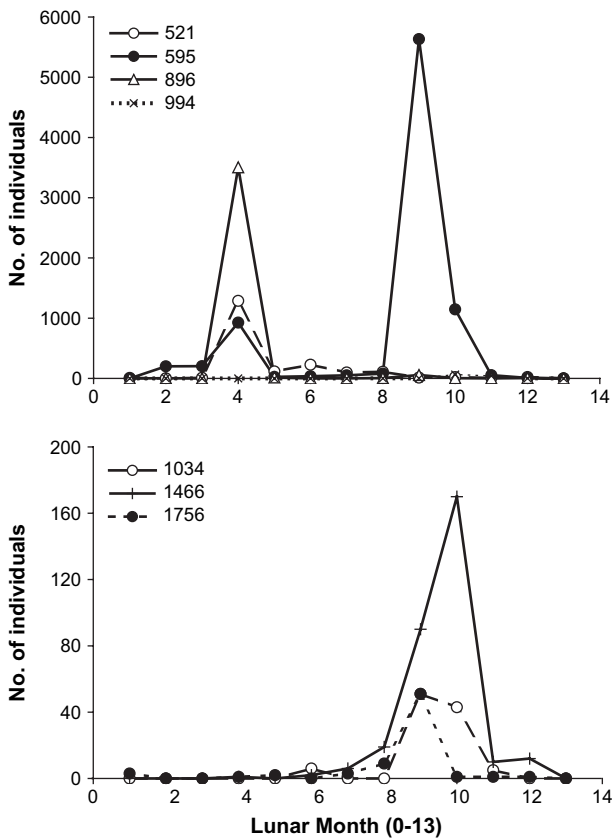


Fig. 2. Abundance of alates (male + female) through time at low (a) and high (b) elevation sites. Each line corresponds to a single elevation. Only sites with more than 30 individuals are shown for clarity. Sites with fewer than 30 individuals show a qualitatively similar pattern. In low elevations, there are two peaks in alate flight, one at the beginning of the summer and one in early to mid fall (autumn). At high elevations, there is a single flight in late fall. Legends show elevations in meters.

Discussion

Although much remains to be learned about the reproductive behavior and ecology of ants, the examination of biweekly collections from 11 sites over 3 years provides a foundation to understand the patterns and constraints on the timing and mode of reproduction in temperate ant assemblages.

With a few interesting exceptions, ant reproductive flights in this temperate system were concentrated in the months from late spring to early fall. In contrast with results from Panama (Kaspari *et al.*, 2001a), but similar to results from the more seasonal Puerto Rico (Torres *et al.*, 2001) and the Polish Carpathians (Woyciechowski, 1987), strong peaks in reproductive flights were detected, with peaks in terms of numbers of individuals during the early and late summer months and a hump shaped pattern of diversity through time, with the most species flying during the end of summer or early fall. Freezing temperatures are common at nearly all elevations throughout the winter in the GSMNP, and such temperatures undoubtedly make reproductive flights in winter unlikely to succeed.

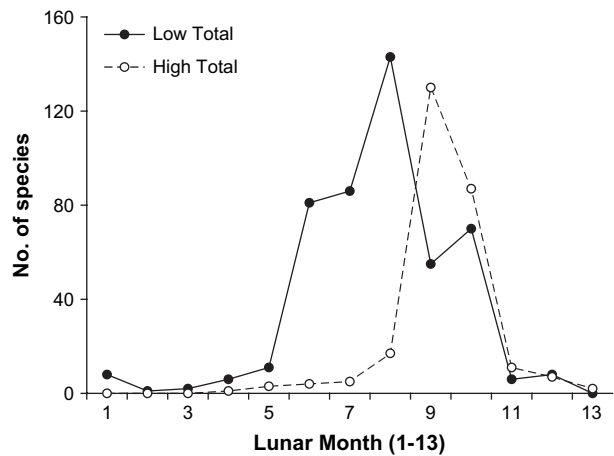


Fig. 3. Alate diversity through time at high and low elevation sites. Diversity peaks earlier at low elevations and is higher throughout, despite there being fewer samples (three sites versus eight) in low elevation sites. Each line shows the pooled results across sites (within elevation category) and year.

Are flights of congeners staggered?

Within those non-winter months in which most species fly, peak flight times of individual species varied greatly and ranged from species such as *Prenolepis imparis* (Say 1836) that flew in early March, not long after the first full low elevation thaw, to species that flew at the end of fall, such as several *Stenamma* and *Myrmica* species. Such differences among species could have evolved to avoid hybridisation between related species. However, the differences in flight times among species were not due to the staggering of flights by congeners. Instead, as in Panama (Kaspari *et al.*, 2001a) and Puerto Rico (Torres *et al.*, 2001), congeneric species tended to fly at similar rather than different times, and differences in flying times were primarily attributable to differences among genera. All *Camponotus* species, for example, tended to fly at the beginning of the summer, whereas *Myrmica* species tended to fly at the end of summer or in early fall. However, information on potential staggering of flights within days is lacking. It is possible that temporal segregation within days, rather than among days, weeks, or months, reduces the probability of hybridisation.

Are flights of species synchronous or asynchronous?

Compared with results from Panama, the flights of the ants of the GSMNP are highly synchronous, even when flights are pooled across several years and a substantial elevation gradient. Within a given year, flights of most species occurred in just one or two months, similar to results for Poland (Woyciechowski, 1987). One possible interpretation of these results is that species with lek mating systems predominate in this assemblage. An alternative hypothesis, however, is that the shorter overall season in this study region relative to Panama (although some species flew in all but lunar month 13, the majority flew between late spring and early fall) and the difficulty of foraging in winter

means that there is strong selective pressure for individuals (or colonies) to fly at the same time.

Contrary to earlier assertions (e.g. Kaspari *et al.*, 2001a) synchrony alone may not be strong evidence for the predominance of lek mating systems in this assemblage. Species likely to be female calling species, including *Myrmecina americana* (Emery 1895), *Ponera pennsylvanica* (Buckley 1866), *Amblyopone pallipes* (Haldeman 1844), and *Hypoponera opacior* (Forel 1893) were not necessarily any more likely to have asynchronous reproductive flights. However, the sex ratios of captured alates of different ant genera add a piece of the puzzle of understanding mating systems. In female calling systems, females do not need to move much or very far, so the majority of ants captured in passive Malaise traps should be males. Five groups of ants showed strongly male-biased sex ratios in the collections (> 90% male, much more biased than the ratios seen in colony collections, e.g. Nonacs, 1986): *Proceratium* species, species of the genera *Ponera* and *Hypoconera* (for which males could not be separated), *Myrmecina americana* (Emery 1895), a monospecific genus in this study region, and *Prenolepis imparis* (Say 1836). Both *Prenolepis imparis* (Say 1836) and *Hypoconera opacior* (Forel 1893) engage in female calling, or at least in mating behaviors in which females exhibit limited movement (Talbot, 1965). Similarly, although the mating biology of *Myrmecina americana* (Emery 1895) has not been documented, the closely related species, *Myrmecina graminicola* (Latreille 1862), is known to engage in female calling (Buschinger, 2003). It seems likely that all those species with disproportionately male-biased collections engage in female calling.

Unlike the timing of peak flights, the synchrony of flights did not appear to be highly conserved from an evolutionary perspective. In ANOVA, neither genus nor subfamily explained significant variation in synchrony. In part, this result may reflect the relatively small variation in synchrony exhibited by the ants in this study region. Alternatively, length of flight period may depend much more on local conditions than on the particular behavior of the species being considered.

Do reproductive flights vary with elevation and among years?

Reproductive flight patterns varied with elevation in a variety of ways. Overall, low elevation sites tended to have species flying mostly in the early summer, or, as at the lowest forest site, in early summer and then again at the end of summer and to a lesser extent in the early fall. In contrast, at higher elevation sites, species tended to fly only at the end of summer and in fall. Thus at low elevations, many species must establish incipient colonies early in the summer which overwinter, whereas at high elevations most species appear to overwinter as solitary queens. The differences among elevations in overall flying times did not result from differences within individual species occurring at both groups of sites, but rather from the absence of those species that fly early in the year at high elevation sites. For those species that did occur at both low and high elevation sites, flight times did differ, as indicated by concordance analyses, but not in consistent directions. Some species flew earlier at high than at low elevations, others flew later, and some species were unchanged in their flight times.

In contrast with the comparisons between high and low elevation sites, patterns of reproduction were relatively consistent among years. At the most coarse scale, species that were early fliers were consistently early fliers, and those that were late fliers were consistently late fliers. Further, the rank order of species flights was similar among years, as indicated by the high values of the concordance analyses. In combination with the above results indicating that flight times are conserved among species within genera, the results of the among-year concordance analyses suggest that when species fly is relatively fixed.

Recent research has revealed how potentially unique the reproductive behavior of particular ant species can be (e.g. Volny & Gordon, 2002; Fournier *et al.*, 2005). So it should not be surprising that comparative analyses of different ant assemblages reveal different patterns among species in terms of reproductive flight phenologies. Overall, this study has shown that the general reproductive flight phenology of a temperate ant assemblage with 57 species differs from an assemblage on Barro Colorado Island in Panama with > 250 species (Kaspari *et al.*, 2001a,b). Clearly much remains to be learned before evolutionary ecologists understand the details of the patterns and constraints on the timing and mode of reproduction for the planet's other 10 000 + ant species.

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