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ANTS IN FLIGHT: REPRODUCTION, DISPERSAL AND PREDATION OF ANT QUEENS

A DISSERTATION APPROVED FOR THE
DEPARTMENT OF BIOLOGY

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Abstract

Flying animals, especially insects, are a prominent feature of most terrestrial environments and make up a majority of the world's known species. The biomechanical demands of flight shape the biology of flying insects at all levels, including their physiology, life history, and interactions with other organisms. Many social insects live in colonies consisting primarily of wingless workers, where the ability to fly is restricted to specialized castes. In most ant species, reproductive queens have wings and fly to leave their nest, mate, and found colonies. Here I investigate the role of flight in ant biology, starting with first principles of flight mechanics and physiology, building to the evolution of different life histories, and finishing with the value of queens as prey for aerial predators, the distribution of ants in the atmosphere, and the spread of invasive species across landscapes.

In Chapter 1 I investigate a potential tradeoff (the Found or Fly hypothesis) that ant queens experience between flight and reproduction. I do this by examining variation in flight morphology within a single species, the tropical cavity-nesting ant, *Azteca instabilis*. Queens of this species vary substantially in abdomen weight throughout the year. Heavier abdomens contain larger nutrient reserves that help young queens rear worker offspring and found new colonies. But they also incur a cost in flight morphology, by reducing flight muscle ratios and increasing wing loading and the drag experienced during flight.

Chapter 2 asks whether the Found or Fly tradeoff applies at an evolutionary level among species. By comparing queens of 21 species from a community in Panama, I find that flight ability is tied to reproductive strategy. Claustral species—those which

fuel colony founding entirely through their abdominal nutrient reserves—have heavier abdomens and lower flight muscle ratios. Claustral species avoid adverse effects on wing loading, however, by evolving larger wings. Several claustral species have flight muscle ratios at or below the theoretical limits for insect flight, and may have adaptations for load-carrying. Queens that have access to external sources of nutrition during colony founding, on the other hand, such as social parasites, fungus-gardeners, and those that hunt their own prey, have lighter abdomens and high flight muscle ratios.

Chapter 3 applies these insights to alternate queen castes within the same species, and attempts to relate flight morphology to performance through a live flight experiment. I examine the flight morphology of two fire ant species that co-occur along the U.S. Gulf Coast—the invasive Red Imported Fire Ant (*Solenopsis invicta*) and the native Tropical Fire Ant (*S. geminata*). In both species, colonies produce both heavy claustral queens and light parasitic queens. Among claustral *S. invicta* queens, individuals with lighter abdomens can fly for longer periods of time. When comparing queen types, claustral queens of both species have heavier abdomens, lower flight muscle ratios, higher wing loading, and higher drag than their parasitic counterparts. In *S. geminata*, claustral queens also have larger wings, offsetting some of the adverse wing loading effects and mirroring interspecific patterns.

The next two chapters address what happens to ant queens once they enter the atmosphere on their mating flights. I attach altitude logging devices to Purple Martins (*Progne subis*), a bird that preys on insects it captures during flight, and monitor the prey they deliver to their young. In so doing I discover (Chapter 4) that Purple Martins in Oklahoma feed primarily on invasive Red Imported Fire Ant queens, and double their foraging efficiency by doing so. I calculate that across the southern USA, Purple Martins probably eat billions of fire ant queens each year. I also measure the flight altitudes of ants and other insects (Chapter 5), and find that heavier ants fly at lower altitudes in the atmosphere, consistent with the Found or Fly tradeoff.

Finally, in Chapter 6 I develop a computer model to simulate the dispersal and reproduction of Red Imported Fire Ants introduced to a novel environment. In the simulations, hypothetical fire ant populations that invest more heavily in parasitic queens, and less in claustral queens, experience larger average colony sizes, higher habitat occupancy, and slower range expansion. When investigating the optimal investment by a reproductive colony, I find that colonies at an expanding range edge benefit more by investing almost entirely in claustral daughter queens, while those in the interior of a range benefit by investing more heavily in parasitic daughters. The two divergent selection regimes may play a role in the evolution of alternate reproductive strategies in ants.

Chapter 1. Found or Fly: nutrient loading of dispersing ant queens decreases metrics of flight ability (Hymenoptera: Formicidae)

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Abstract

Young ant queens face two conflicting challenges. First, they must fly to mate, disperse and locate a nest site. Second, they must found a new colony and raise their first workers with their own nutrient reserves. The Found or Fly (FoF) hypothesis posits a fitness tradeoff between colony founding success and flight ability, mediated through abdominal nutrient loading of young queens. It proposes that though heavier abdomens increase survival during the founding period, they do so at the expense of a queen's ability to mate, disperse, and survive the mating flight. We evaluate FoF by characterizing the flight morphology of a common Neotropical year round breeder, *Azteca instabilis* (SMITH, 1862). Abdomen mass varied among queens independently of body size and throughout the year. Heavier abdomens adversely impacted three metrics of flight ability: flight muscle ratio, wing loading and drag. These patterns are consistent with FoF. FoF links reproductive demands, morphology and dispersal ability, and provides a quantitative framework for understanding dispersal variation across the ants. FoF provides insight into several areas of ant ecology and evolution, including alternative reproductive strategies, sexual dimorphism and invasions.

Introduction

Flight is a key evolutionary development of the Insects (Wagner & Liebherr 1992; Dudley 2000; Gullan & Cranston 2010) but its specialized physiological and morphological requirements (Ellington 1984; Norberg & Rayner 1987; Rayner 1988; Dudley 2000) constrain many aspects of insect biology. Ants (Formicidae]) are no exception. The flight phase of an ant's life, however, is brief and may be as short as one half hour for some queens (Markin et al. 1971; Hölldobler & Wilson 1990). During this time a young queen must perform the vital tasks of mate location, sex, dispersal and nest site location (Hölldobler & Wilson 1990; Peeters & Ito 2001). Flight exposes ants to environmental hazards (Hölldobler & Wilson 1990; Nichols & Sites 1991; Peeters & Ito 2001; Fjerdingstad & Keller 2004) and mortality can be as high as 99% (Gordon & Kulig 1996), making it the deadliest phase in the life cycle. The combination of reproductive consequences and mortality suggests that ant queens experience strong selection during flight (Buschinger & Heinze 1992; Fjerdingstad & Keller 2004). At the same time, flight is the least understood part of the colony life cycle, with investigations of reproduction mostly limited to colony foundation and early growth (e.g. Tschinkel 1993a; Peeters & Ito 2001).

Flight precedes colony foundation, when a young queen sheds her wings, initiates oogenesis and lays her first eggs (Keller & Passera 1988; Tschinkel 1988c; Hölldobler & Wilson 1990; Peeters & Ito 2001). Producing eggs and rearing the first cohort of workers requires substantial energy reserves—up to over 60% of queen body mass (Peakin 1972; Keller & Passera 1989)—especially for the majority of species whose queens do not forage (Toom et al. 1976; Voss & Blum 1987; Keller & Passera 1989; Keller & Ross 1993a; DeHeer et al. 1999; Johnson 2006). Mature queens develop abdominal reserves of fats and storage proteins by pre-flight feeding in their natal colony (Peakin 1972; Boomsma & Isaaks 1985; Nielsen et al. 1985; Keller & Passera 1989; Martinez & Wheeler 1994; Hahn et al. 2004), and histolysis of flight muscles after wing loss provides a supplementary protein source (Hölldobler & Wilson 1990; Wheeler & Martinez 1995; Wheeler & Buck 1996; Peeters & Ito 2001; Brown & Bonhoeffer 2003). Heavier abdomens store more energy and increase colony founding success by increasing the rate (Wagner & Gordon 1999) or amount (Tschinkel 1993a; Liu et al. 2001; DeHeer 2002) of early offspring production, and increasing survival during the founding period (Mintzer 1987; Nonacs 1992; Balas & Adams 1996; Bernasconi & Keller 1996; Johnson 1998; Bernasconi & Keller 1999; Adams & Balas 1999; Johnson 2001).

At the same time, these weight increases, which can be up to 290% of a queen's body mass (Boomsma & Isaaks 1985), likely impact flight ability and thereby incur fitness costs by reducing mating success (Davidson 1982; Fjerdingstad & Boomsma 1997; Wiernasz et al.1995; Vogt et al. 2000; Wiernasz & Cole 2003), dispersal distance (Fortelius et al. 1987; Sundström 1995; Zera & Denno 1997; Rüppell et al. 1998; Lachaud et al. 1999; Gu et al. 2006) and predator evasion (Fjerdingstad & Keller 2004). Several metrics of insect flight ability are tied to abdomen mass. Heavier abdomens adversely impact flight muscle ratio and wing loading (Hedenström 1992; Marden 1987, 2000; Dudley 2000). Nutrient loading may also increase drag by altering abdomen shape (Dudley 2000). These changes alter flight speed and reduce maneuverability, maximum flight time and overall flight performance (Norberg & Rayner 1987; Rayner 1988; Hedenström 1992; Dudley 2000; Marden 2000; Vogt et al.

2000). The opposing effects of abdomen mass thus suggest that constraints associated with flight may limit founding performance.

We summarize this situation in what we call the Found or Fly (FoF) Hypothesis, which posits a fitness tradeoff between colony founding success and flight ability mediated by abdomen mass. FoF has three assumptions: A1) queen abdomen mass is a plastic trait that varies with feeding behavior or food availability; A2) heavier abdomens increase founding success; and A3) heavier abdomens decrease flight ability. A tradeoff between colony founding and flight ability has long been recognized in the context of the evolution of flightless or nondispersing queens (Winter & Buschinger 1986; Buschinger & Heinze 1992; Tinaut & Heinze 1992; Sundström 1995; Heinze & Tsuji 1995; McInnes & Tschinkel 1995; Rüppell et al. 1998; Rüppell & Heinze 1999; Lachaud et al. 1999; Heinze & Keller 2000; Peeters & Ito 2001; Steiner et al. 2006; Peeters 2012; Peeters et al. 2012). FoF, however, explicitly recognizes variation in flight ability among dispersing queens and thereby extends this tradeoff to all ants, highlights morphological links between ecology and reproductive strategy, and provides a framework for quantifying variation in dispersal ability.

Although the role of abdomen mass in colony founding (A2) is well documented, its effects on flight ability are poorly understood. Here we evaluate the remaining assumptions of FoF—abdomen mass variation (A1) and its relationship to flight ability (A3)—by examining the flight morphology of *Azteca instabilis* (Smith, 1862) (Dolichoderinae), a common Central American species. *Azteca instabilis* is a habitat generalist with a representative life cycle in which young queens go on mating flights and found new colonies in hollow tree trunks (Longino 2010). Rather than having a pulsed mating season where queens fly for only a brief period each year, *A. instabilis* queens fly and mate year round (Kaspari et al. 2001a, b), providing an ideal system for examining variation in nutrient loading and flight ability. Queen mass of temperate seasonal maters may vary between flights or with food supply (e.g. Tschinkel 1993a; Ode & Rissing 2002; Fjerdingstad & Keller 2004), but we know of no studies of annual variation in queen investment from a tropical species. Using *A. instabilis* as a model we document how abdomen mass variation affects three flight ability metrics, and explore how abdomen investment can mediate a tradeoff between founding and flight.

Materials and methods

We collected alate queens during their mating flights on Barro Colorado Island, Panama (9°9’19’’N, 79°50’15’’W), a lowland seasonally wet forest. Two modified Pennsylvania black-light traps were hung from the canopy on a ridge 120 m a.s.l, three and 27 m above ground level (Kaspari et al. 2001a, b). Traps were run continuously and checked weekly for one year beginning in mid June 1991. Ants were initially preserved in 70% ethanol and then transferred to 95% ethanol. *Azteca instabilis*, an abundant generalist and year round flyer (Kaspari et al. 2001a, b) with a representative life cycle, was chosen as a model to evaluate FoF. Queens are larger than workers, with a queen to worker head width ratio of 1.34 (Longino 2007), are believed to found colonies claustrally in hollow tree trunks (Longino 2010), and may fly long distances to find suitable nest sites (Bruna et al. 2011).

Ninety queens from throughout the year were selected for morphological analysis. To ease comparison with other insects, we here use the word “abdomen” to refer to what is properly called the “gaster,” and “thorax” in place of “mesosoma.” Linear measurements—head width, abdomen length and abdomen height—were made to 0.1 mm with an ocular micrometer under a dissecting microscope. Head width, a standard measure of body size, is the maximum width of the head in full-face view, excluding the eyes. Abdomen length is here defined as the maximum linear measurement of the abdomen from the dorsal point of attachment of the petiole. Abdomen height was measured as the maximum vertical measurement of the abdomen when oriented horizontally in lateral view. After linear measurements the wings, legs, abdomen and head were removed with surgical scissors, keeping the thorax and petiole intact, and all parts dried at 60-65 ˚C for 48 hours. The abdomen, hindwings, forewings, thorax+petiole, and entire body were weighed to the nearest 0.001 mg with a Cahn microbalance. Storage in alcohol may reduce specimen dry mass (Porter 1992), adding some noise to the mass data. Mass loss per se would not add bias, but because fat is slightly soluble in ethanol, fatter queens may lose more mass during storage than thinner ones. This would reduce the observed differences between queens, adding a conservative bias, if any. Finally, we made wing measurements for each specimen. We made slides of one forewing and one hindwing from each queen and photographed them with a reference ruler using a Leica dissecting microscope camera. ImageJ software (Schneider et al. 2012) was used to measure the lengths and areas of the forewing and hindwing. After processing one individual was found to be a different species and was removed from analysis, and one record was removed as an outlier. Several specimens that had dried during storage in ethanol showed anomalous mass measurements and were removed from analysis. Ultimately, 73 individuals were analyzed of the 90 processed, collected from 26 weeks of the year. Some analyzed individuals lacked measurements due to missing or damaged body parts. We excluded those individuals when relevant and note the sample size for each analysis.

We tested the assumption (A1) of abdomen mass plasticity in two ways. First, to see if abdomen investment varies independently of intrinsic body size we compared abdomen mass to head width. Second, we compared weekly samples throughout the year with a Kruskal-Wallis test (Sokal & Rohlf 1995) to see whether abdomen mass varies over time. Seasonal changes in environmental conditions are one possible source of temporal variation in abdomen mass. To examine whether abdomen mass changes in response to seasonal environments, we compared abdomen mass between the wet and dry seasons. The less productive dry season on Barro Colorado Island lasts approximately from January 1 to May 1 (Leigh et al. 1996) and corresponds to weeks 1 through 17.

To evaluate how heavier abdomens affect flight ability (A3) we compared three standard morphological metrics—flight muscle ratio, wing loading and drag reference area—to abdomen mass. Flight muscle ratio (FMR), the ratio of flight muscle mass to body mass, may be the most important predictor of insect flight ability (Marden 1987, 2000, Dudley 2000). FMR is proportional to acceleration and load lifting ability and a higher FMR increases maneuverability and flight endurance. FMR was calculated by dividing thorax+petiole mass by total body mass. While not a direct measure of flight muscle, the thorax consists predominantly of flight muscle and thorax mass is often used as a surrogate for flight muscle in insects, including ants (Fjerdingstad & Boomsma 1997; Van Dyck & Matthysen 1999; Norberg & Leimar 2002; Dillon &

Dudley 2004; Darveau et al. 2005; Merckx & Van Dyck 2006). The petiole was left attached for practical reasons, and is unlikely to affect the results because it is small compared to other body parts, especially the thorax. Using thorax+petiole mass as a surrogate for flight muscle slightly overestimates FMR, introducing a conservative bias, as it might mask reductions in FMR with increasing abdomen mass.

Wing loading (Nm^{-2}), the ratio of body weight to wing area, is negatively related to maneuverability, flight endurance and maximum flight speed, and positively related to minimum power and speed requirements for flight (Norberg & Rayner 1987; Rayner 1988; Hedenström 1992; Dudley 2000; Vogt et al. 2000; Darveau et al. 2005). To calculate wing loading, body mass was divided by the total area of all four wings and converted to $Nm\text{-}^{2}$.

Drag, proportional to a cross sectional reference area, decreases overall flight performance (Dudley 2000). To assess changes in abdomen drag with nutrient loading we used a volumetric reference area, $V^{2/3}$ (mm^2), a biologically relevant measure that links mass and shape (Alexander 1990; Vogel 1994). We calculated abdomen volume with the formula for a prolate spheroid, using abdomen length as the major axis and abdomen height as the minor axis, and raised the resulting volume to the 2/3.

To further characterize flight morphology two wing characters—aspect ratio and wing mass density—were calculated and compared to abdomen mass. Aspect ratio, defined here as (4 x forewing length^2)/total wing area, is a measure of wing shape. Narrower wings have higher aspect ratios and increased aerodynamic efficiency (Norberg & Rayner 1987; Rayner 1988; Dudley 2000). Wing mass density (mg/mm^2) is a measure of wing stiffness and durability, calculated by dividing the total wing mass by total wing area. Although we measured aspect ratio and wing mass density as part of a general characterization of flight morphology, we didn't expect abdomen mass to affect either measure since they are developmentally determined wing traits and unrelated to plastic changes in abdomen mass.

Before comparing flight metrics to abdomen mass we checked each for a relationship with head width to correct for body size. FMR, wing loading and aspect ratio were not related to head width. Drag and wing mass density increased with head width. In those cases the residuals from the regression versus head width were plotted against abdomen mass.

All statistics were performed in R (R Core Team 2012). Variables were visually examined for normality by plotting. Regressions were standardized major axis regressions using the "lmodel2" package (Legendre 2011) to account for measurement error of independent variables (McArdle 1988). To calculate residuals of regressions against head width ordinary least squares regression was used, which is more appropriate for prediction (Sokal & Rohlf 1995). Regressions of flight metrics against abdomen mass were tested for heteroscedasticity using the "car" package (Fox & Weisberg 2011). To account for experimentwise error we applied the Holm-Bonferroni correction (Holm 1979) to *p*-values of regressions of flight metrics against abdomen mass.

Results

We analyzed 73 queens from 26 weeks of the year that varied over 1.5 fold in body mass and 1.2 fold in linear body size. Body mass averaged 21.7 (±2.2, n = 66) mg, average abdomen mass was 14.2 (±1.9, n = 73) mg, and average head width was 2.4

(±0.09, n = 73) mm. Abdomen mass varied 1.8 fold and was unrelated to head width ($r^2 = 0.02$, $P > 0.2$, n = 73), indicating that the nutrient load of a queen is unrelated to her intrinsic body size. For example, the entire observed range of abdomen mass values, from 10.4 to 18.6 mg, were associated with the modal head width of 2.4 mm. Median abdomen mass of queens varied weekly throughout the year (Kruskal-Wallis, $P = 0.03$, Figure 1) from a low of 10.7 mg in week 22 to a high of 16.0 mg in week 12 (low and high from weeks with ≥ 3 queens), but was the same over the more productive wet and less productive dry seasons (medians = 14.5 vs. 14.4 mg, respectively, Kruskal-Wallis, $P > 0.9$). Variation in abdomen mass among queens and from week to week, unrelated to variation in intrinsic body size, supports the assumption (A1) that abdomen mass is a plastic trait.

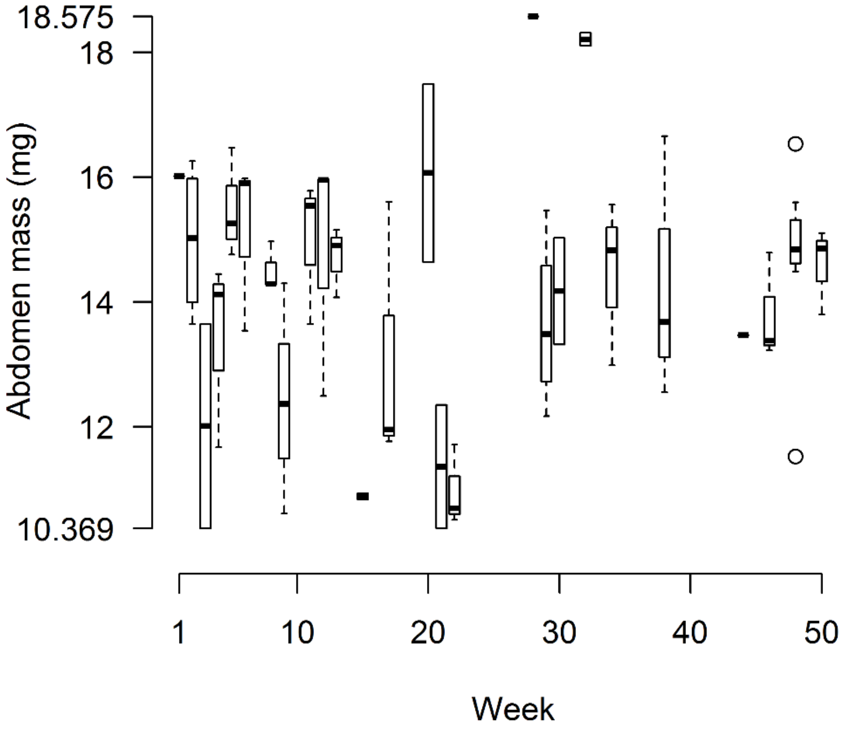

Figure 1. Abdomen mass of young queens varies weekly throughout the year (n = 73, Kruskal-Wallis, P = 0.03), reflecting the plasticity of this trait. Weeks 1 through 17 correspond to the dry season on Barro Colorado Island. Box plots show medians, quartiles and outliers. Weeks 1, 28 and 44 have only one measured queen.

Heavier abdomens adversely impacted all three metrics of flight ability. As expected, the two wing characters, aspect ratio (mean 6.3 ±0.3, n = 43) and wing mass density (mean 0.0041 ±0.0006 mg/mm^2, n = 43), were invariant with abdomen mass. Flight muscle ratio (mean 0.20 ±0.021) decreased over 30% from the lowest to highest abdomen mass ($P < 0.03$, Figure 2A). Wing loading (mean 2.67 ±0.23 Nm^{-2}) increased about 40% over the range of abdomen mass ($P < 0.03$, Figure 2B). For drag reference area (mean 10.11 ±1.5 mm^2), size-corrected values increased about 2 mm^2 with abdomen mass ($P < 0.03$, Figure 2C). Increased abdomen investment, based on these morphological metrics, likely reduces maneuverability and flight endurance, and increases power requirements, supporting the assumption (A3) that heavier abdomens decrease flight ability.

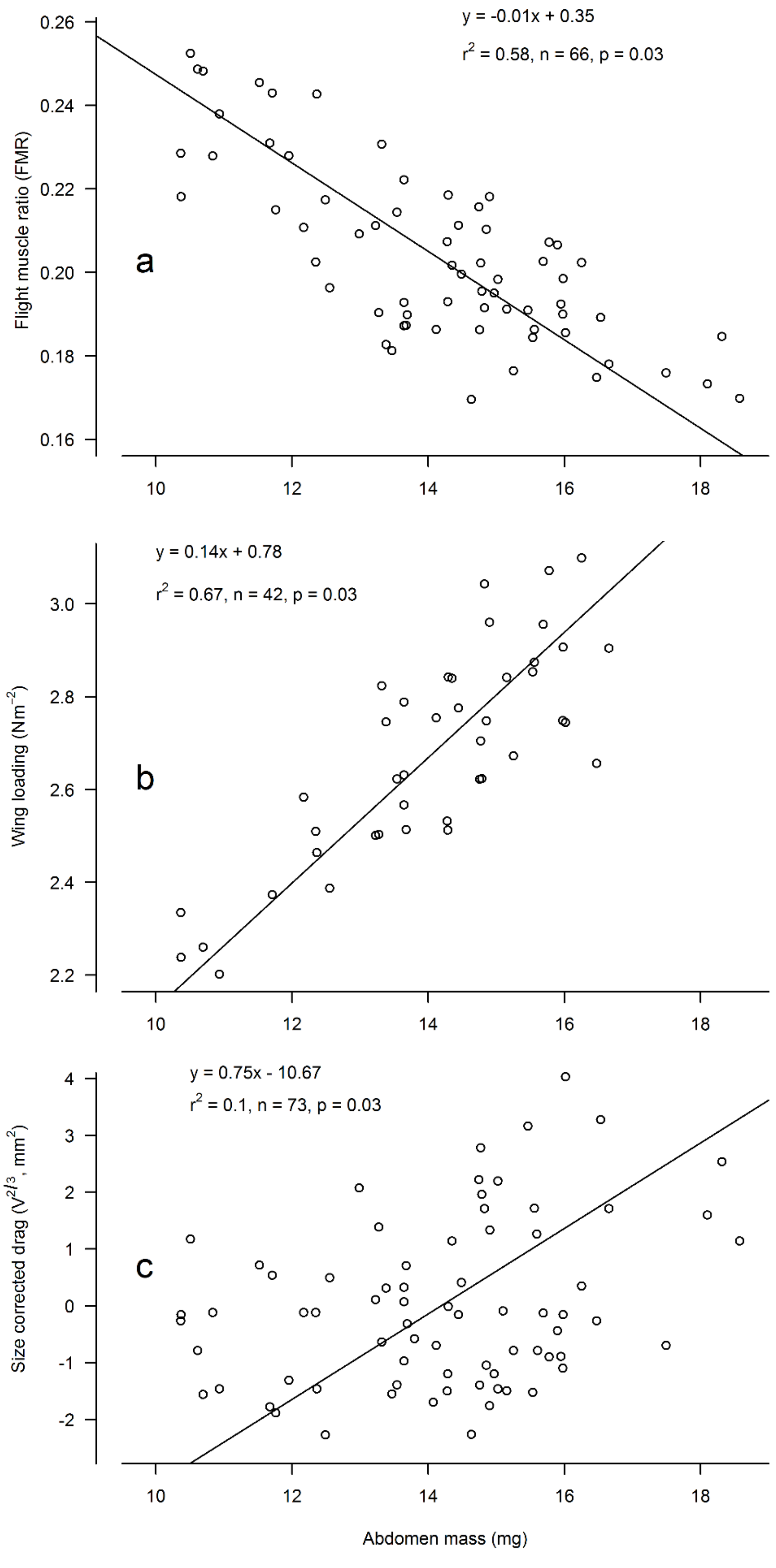
y = -0.01x + 0.35
r^2 = 0.58, n = 66, p = 0.03
a
Flight muscle ratio (FMR)
0.26
0.24
0.22
0.20
0.18
0.16
y = 0.14x + 0.78
r^2 = 0.67, n = 42, p = 0.03
b
Wing loading (Nm^{-2})
3.0
2.8
2.6
2.4
2.2
y = 0.75x - 10.67
r^2 = 0.1, n = 73, p = 0.03
c
Size corrected drag ($V^{2/3}$, mm^2)
4
3
2
1
0
-1
-2
10
12
14
16
18
Abdomen mass (mg)

Figure 2. Nutrient loading adversely impacts flight ability metrics. a) Flight muscle ratio declines with nutrient loading. Reduced FMR decreases maneuverability, flight endurance, acceleration and load lifting ability. b) Wing loading increases with nutrient loading. Higher wing loading reduces maneuverability, maximum time aloft and maximum flight speed, and increases minimum speed and power required for flight. c) Drag increases with nutrient loading. $V^{2/3}$ is proportional to drag, which reduces overall flight performance. Drag values are residuals from OLS regression on head width.

Discussion

The Found or Fly (FoF) hypothesis extends a recognized tradeoff between founding and dispersal in the evolution of flightless queens (e.g. Sundström 1995; Heinze & Keller 2000), to posit a fitness tradeoff between colony founding and flight success among flying queens, mediated by abdomen investment. Using a common Neotropical species as a model, we provide the first comprehensive characterization of an ant's flight morphology. In doing so, we document 80% variation in abdomen investment among queens, with commensurate variation in flight ability metrics. This relationship between abdomen investment and flight morphology establishes a framework with potential for understanding dispersal variation across the ants.

The observed range of abdomen masses has several consequences for flight and reproduction. Comparing hypothetical queens with abdomen masses of 10.5 and 18.5 mg, corresponding to total body masses of 17.5 and 25.5 mg, the heavier queen would have 2/3 the flight muscle ratio and 1.5 times the wing loading of the lighter, and experience higher abdomen drag. As a rough approximation, the lighter queen therefore can be expected to lift 1.5 times as much weight as the heavier (Marden 1987), accelerate 1.5 times as quickly (Marden 2000), take turns 33% more sharply or 1.2 times as fast (Marden 1987, 2000), fly for longer periods of time (Marden 2000), have lower wingbeat frequencies and metabolic demands (Darveau et al. 2005), and be able to fly both faster (Vogt et al. 2000) and 18% slower than the heaviest queens (Norberg & Rayner 1987). Improved maneuverability, flight endurance and flight speed range suggest that lighter queens are better able to locate and choose mates, mate aerially, escape predators, disperse farther and find suitable nest sites. The heavier queen, on the other hand, if she invests all the extra weight into offspring production, would be able to produce more offspring more quickly during the founding period. In the fire ant *Solenopsis invicta* a hypothetical difference of 8 mg dry weight, assuming a live to dry weight ratio of 2 (Tschinkel 1993a), could be expected to produce 65 to 90 more initial workers (Tschinkel 1993a; DeHeer 2002). Similarly, in the harvester ant *Pogonomyrmex barbatus* the faster egg production associated with a difference of 8 mg dry weight would mean attaining maximum egg production 6 days sooner (Wagner & Gordon 1999). Although these cost and benefit estimates are necessarily crude, they illustrate the fitness tradeoffs queens and colonies face when loading nutrients.

Two issues arise as to whether abdomen mass is a suitable surrogate for nutrient investment. First, heavier abdomens may result from flight fuel loading rather than nutrient investment for colony founding. Like other hymenopterans, ants use glycogen as flight fuel, not fats (Beenakkers 1969; Toom et al. 1976; Jutsum & Quinlan 1978; Passera & Keller 1990; Passera et al. 1990; Vogt et al. 2000). Glycogen storage is not restricted to the abdomen, makes up only a small percentage of body mass (1-10%), and is quickly depleted during flight (Toom et al. 1976; Passera & Keller 1990; Passera et al. 1990; Sundström 1995). Conversely, abdominal fat alone can comprise the majority of a queen's body mass (Keller & Passera 1989) and is not used in flight. We are therefore confident that variation in abdomen mass, especially among queens captured in flight, accurately captures variation in nutrient reserves.

Second, some nutrients are stored in the thorax. Queens histolyze their flight muscles after wing loss, providing a supplemental nutrient source during colony founding (Hölldobler & Wilson 1990). Most of the energy and amino acids used in colony founding, however, come from abdominal fats and storage proteins, with flight muscle only of secondary importance (Wheeler & Martinez 1995; Wheeler & Buck 1996; Brown & Bonhoeffer 2003). While species that rear offspring entirely with their own nutrient reserves are believed to have absolutely larger flight muscles, these function mainly to carry the extra abdominal loading (Peeters & Ito 2001), and are actually smaller relative to total body mass (Jackson Helms & Mike Kaspari, unpublished data). At any rate, queens don’t adjust flight muscle content in preparation for colony founding and differences in nutrient loading among polymorphic queens are reflected in abdomen mass rather than thorax mass variation (e.g. Keller & Ross 1993a, b).

FoF makes predictions about a variety of phenomena associated with the brief but critical flight phase. For example, we expect queens practicing reproductive strategies with different nutrient demands (Keller & Passera 1989) to vary predictably in flight and dispersal ability. Similarly, we expect male abdomen size and flight ability to vary with mating strategy (Davidson 1982) and sperm load (Fjerdingstad & Boomsma 1997). Better dispersal ability associated with low levels of queen abdomen investment (Keller & Ross 1993a, b; Yamauchi & Ogata 1995; Rüppell & Heinze 1999) may even contribute to a species’ invasiveness. Incorporation of flight into our understanding of the ant life cycle promises to shed light on numerous aspects of ant ecology and evolution, including alternative reproductive strategies, sexual dimorphism, population dynamics, gene flow and conservation.

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Chapter 2. Reproduction-dispersal tradeoffs in ant queens

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Abstract

Organisms often experience reproduction-dispersal tradeoffs mediated by body size. In ants (Hymenoptera: Formicidae) the Found or Fly (FoF) Hypothesis states that dispersing queens face an ecological tradeoff between colony founding and flight success mediated by abdominal nutrient loading. If expressed interspecifically, such a tradeoff implies biomechanical costs to more energetically demanding life history strategies. Claustrally founding queens, who carry the entire resource load necessary to fuel early colony growth, may incur flight costs. We characterized the flight morphology of 21 Neotropical species representing four major subfamilies, spanning four orders of magnitude in body mass and practicing several colony founding strategies. Flight morphologies were compared in a phylogenetic context to evaluate how they varied with body size and reproductive ecology. Consistent with FoF, claustral founders had 30% lower flight muscle ratios (FMR) and trended toward higher abdomen drag than species in which founding queens feed. The two strategies did not differ in wing loading. Instead, claustral founders evolved larger wings, counteracting the effect of heavier abdomens. Heavy nutrient loads pushed several claustral species to theoretical limits of flight by lowering FMR to levels which cause flightlessness in other insects. Selection for higher nutrient loads related to colony founding is a possible mechanism for the recurrent evolution of flightlessness in ants. The importance and conflicting demands of nutrient storage and flight make ant queens ideal organisms for modeling reproduction-dispersal tradeoffs. By emphasizing the role of flight in ant biology, the FoF Hypothesis highlights this tradeoff and provides novel insights into ant evolution.

Introduction

Aerially dispersing plants and insects often experience reproduction-dispersal tradeoffs, in which higher nutrient loads increase reproductive or competitive ability at the expense of flight or dispersal (Harrison 1980; Guries and Nordheim 1984; Wagner and Liebherr 1992; Zera and Denno 1997; Marden 2000). Though worker castes are wingless, most of the world’s more than 12,000 ant species (Bolton et al. 2006) rely on flight for dispersal and reproduction (Hölldobler and Wilson 1990; Peeters and Ito 2001). Virgin queens and males fly to disperse, find partners and mate, after which queens found new nests (Hölldobler and Wilson 1990). Flight is the deadliest phase of the life cycle because it exposes queens to predators and other hazards (Hölldobler and Wilson 1990; Nichols and Sites 1991; Peeters and Ito 2001; Fjerdingstad and Keller 2004; Frederickson 2006), and over 99% may die during this brief window (Gordon and Kulig 1996). The role of flight in the colony life cycle and its associated biomechanical constraints (Ellington 1984; Wagner and Liebherr 1992; Dudley 2000) and high mortality combine to drive queen evolution through flight related selection (Buschinger and Heinze 1992; Wiernasz and Cole 2003; Fjerdingstad and Keller 2004; Keller et al. 2014). But the brevity of this phase—much less than 1% of a queen's life (Hölldobler and Wilson 1990)—has resulted in comparatively little study of ant flight (but see Markin et al. 1971; Vogt et al. 2000).

Ant queens are analogous to plant seeds—nutritive propagules that disperse to found sessile colonies—and experience similar tradeoffs (Andersen 1991; Johnson 1998). Ant queens build up fat and protein reserves in their abdomens by feeding before flying from their natal nests (Peakin 1972; Boomsma and Isaaks 1985; Nielsen et al. 1985, Keller and Passera 1989; Martinez and Wheeler 1994). After arrival at a new nest site, a heavier abdomen increases a queen's reproductive output and survival until she rears enough workers to function as a colony (Mintzer 1987; Nonacs 1992; Tschinkel 1993a; Balas and Adams 1996; Bernasconi and Keller 1996, 1999; Johnson 1998; Adams and Balas 1999; Wagner and Gordon 1999; Johnson 2001; Liu et al. 2001, DeHeer 2002). At the same time, heavier abdomens reduce metrics of flight ability by decreasing flight muscle ratio (FMR) and increasing wing loading and drag (Helms and Kaspari 2014). These changes likely reduce a queen's dispersal distance (Fortelius et al. 1987; Sundström 1995; Rüppell et al. 1998; Lachaud et al. 1999), ability to evade predators (Fjerdingstad and Keller 2004), and success in aerial mate choice and copulation (Davidson 1982; Fjerdingstad and Boomsma 1997; Wiernasz et al. 1995; Vogt et al. 2000; Wiernasz and Cole 2003). The Found or Fly (FoF) Hypothesis (Helms and Kaspari 2014) proposes that individual queens experience an ecological fitness tradeoff between colony founding and flight mediated by abdominal nutrient loading. It remains to be seen, however, whether such a tradeoff is expressed interspecifically.

Nutrient loads are key variables in the various reproductive strategies of ants (Keller and Passera 1989). The predominant strategy, used by a vast majority of species, is claustral founding, in which a newly mated queen seals herself in a new nest and rears workers off her body reserves (Hölldobler and Wilson 1990; Brown and Bonhoeffer 2003; Keller et al. 2014). Claustral founding requires abdominal reserves ranging from 40% to over 60% of queen body weight (Keller and Passera 1989). Many species, however, use an ancestral strategy in which queens leave the incipient nest to forage (Johnson 2002; Brown and Bonhoeffer 2003). Other queens may feed on symbiotic fungi (Seal 2009) or insects (LaPolla and Spearman 2007), join existing colonies or parasitize host species (Buschinger 1986, 2009; Keller and Passera 1989; Hölldobler and Wilson 1990), or are accompanied by workers from their natal nest (Cronin et al. 2013). These non-claustral strategies require lighter nutrient loads (Keller and Passera 1989). The resulting variation in abdomen weight, by altering flight morphology, should generate flight differences among life histories, although we know of no such analyses.

The FoF Hypothesis states that the heavier abdomens of claustral founders should reduce flight ability metrics relative to non-claustral species. We predict claustral founders will have (P1) lower FMRs, (P2) higher wing loading and (P3) higher abdomen drag. If the FMR drops too low a queen loses the ability to fly. Abdomen weights which lower FMR to this marginal value—from 0.12 to 0.16 in insects (Marden 2000)—represent the maximum load a queen can carry and still disperse by flight. We therefore predict (P4) claustral founders, for whom energy storage is paramount, will approach these marginal values. Evolutionary events, on the other hand, may allow species to break tradeoffs that apply to individuals. For example, insect wings can be evolutionarily labile (Mezey and Houle 2005), and increases in wing loading—the ratio of body weight to wing area—can be offset by evolving larger wings at little energetic cost. It is less clear whether tradeoff breaking is possible for FMR and drag effects, as it would require relatively expensive or complex changes in thorax investment or abdomen density.

The FoF model laid out above differs from previous analyses of queen life history evolution. For example, claustral founders have been predicted to have larger flight muscles so that muscle histolysis can help fuel colony founding (e.g. Hölldobler and Wilson 1990; Peeters and Ito 2001). Moreover, other models view the evolution of claustral founding only in terms of the costs and benefits of foraging mortality and energy provisioning (Hölldobler and Wilson 1990; Brown and Bonhoeffer 2003), and ignore the costs and benefits of dispersal. FoF provides a potentially useful alternative by considering the impacts of heavier abdomens on flight.

To contrast these approaches in the study of queen biology, we characterize the flight morphology of 21 co-occurring Neotropical species to explore the role of flight in queen evolution. The species represent four major subfamilies, display a variety of reproductive strategies and span four orders of magnitude in body mass, approximating the entire range of variation among flying queens. We examine how flight morphology scales with body size in a phylogenetic context, compare reproductive strategies to evaluate predictions of FoF, and look for evidence of evolutionary compensation for heavier abdomens. By recognizing flight mechanics as a driver of ant biology we generate novel predictions and suggest a previously unrecognized life history tradeoff—the dispersal cost of claustral founding.

Materials and methods

Specimen collection

All specimens were collected in 1991-1992 in a lowland seasonally wet forest on Barro Colorado Island, Panama (9°9'19"N, 79°50'15"W). Alate queens were captured during their mating flights in black-light traps and preserved in ethanol (Kaspari et al. 2001a, b). Among the species captured, we selected 21 for analysis. Species were chosen to span the entire natural range of flying queen body size, represent all four major subfamilies, and capture a variety of reproductive strategies. Although we generally chose only one species per genus, to capture intrageneric variation we analyzed multiple species for two genera, Dolichoderus and Camponotus. Some of our data for one species, Azteca instabilis, were previously published in a separate study (Helms and Kaspari 2014). Queens within a species vary substantially in flight morphology (Helms and Kaspari 2014), but pilot studies showed that variance of most measures stabilized after measuring three to six individuals. We thus attempted to analyze at least six individuals per species but due to limited availability we used smaller sample sizes for some (Table 1).

Flight morphology

We characterized the flight morphology of each specimen according to the protocol outlined in Helms and Kaspari (2014). For simplification, we refer to the gaster as the “abdomen” and the mesosoma as the “thorax.” We measured head width, abdomen length and abdomen height to 0.1 mm with an ocular micrometer under a dissecting microscope. Head width is the maximum width of the head in full-face view excluding the eyes and is a standard measure of ant body size. We removed the wings, legs, abdomen and head, keeping the thorax and petiole or post-petiole intact, and dried all parts at 60-65 °C for 48 hours. Unlike the other species the post-petiole of Atta colombica is large and broadly attached to the abdomen. In that case the petiole was left attached to the thorax, but the post-petiole was kept with the abdomen. Due to their large size Atta specimens were dried for 72 hours. After drying we weighed the forewings, hindwings, abdomen, thorax+petiole/post-petiole, and entire body to the nearest 0.001 mg with a Cahn microbalance. Although storage in alcohol may reduce dry mass (Porter 1992) we do not suspect the introduction of bias, as all specimens were preserved similarly. After weighing we made slides of a forewing and hindwing from each queen and photographed them with a reference ruler using a Leica dissecting microscope camera or digital camera, depending on the size of the species. We measured the lengths and areas of the forewing and hindwing using ImageJ software (Schneider et al. 2012).

After measurements we calculated flight morphology metrics for each specimen. Flight muscle ratio is the ratio of flight muscle mass to body mass and may be the most important predictor of insect flight ability (Marden 1987, 2000; Dudley 2000). FMR is proportional to acceleration and load lifting ability, and a higher FMR increases maneuverability, flight endurance and the range of temperatures at which an insect can fly. We calculated FMR by dividing the thorax+petiole/post-petiole mass by total body mass. The thorax is mostly flight muscle and thorax mass is a standard surrogate for flight muscle in insects, including ants (Fjerdingstad and Boomsma 1997; Van Dyck and Matthysen 1999; Norberg and Leimar 2002; Dillon and Dudley 2004; Darveau et al. 2005; Merckx and Van Dyck 2006). The petiole and post-petiole—narrow segments connecting the thorax and abdomen—were left attached for practical reasons. Although the petiole and post-petiole are small and unlikely to affect our results, it is worth noting that including them in the thorax mass slightly overestimates FMR. To calculate wing loading—the ratio of body weight to wing area—we divided body weight by the total area of all four wings (Nm^{-2}). Wing loading is negatively related to maneuverability, flight endurance and maximum flight speed, and positively related to minimum power and speed requirements for flight (Norberg and Rayner 1987; Rayner 1988; Hedenström 1992; Dudley 2000; Vogt et al. 2000; Darveau et al. 2005). Drag decreases overall flight performance (Dudley 2000) and is proportional to a cross sectional reference area. We are primarily interested in abdomen drag and used a volumetric reference area, abdomen volume$^{2/3}$ (mm^2), which intuitively links mass and shape (Alexander 1990; Vogel 1994). To calculate volume we treated the abdomen as a prolate spheroid with abdomen length and height as the major and minor axes. Wingspan is the combined length of both forewings, and wing area is the combined area of all four wings. Aspect ratio is a measure of wing narrowness and is calculated as (4 x forewing length^2)/total wing area. Higher aspect ratios—narrower wings—increase aerodynamic efficiency (Norberg and Rayner 1987; Rayner 1988; Dudley 2000). To calculate wing mass density—a measure of wing stiffness and durability—we divided total wing mass by total wing area (mg/mm^2).

Reproductive strategy

We assigned each species a reproductive strategy based on literature and information on AntWeb (AntWeb 2013). We use the term claustral founding to refer to strategies in which the queen does not feed during colony founding and fuels early colony growth entirely off her body reserves. We use the term non-claustral founding to refer to all strategies in which the queen is likely to feed during the founding process, either through foraging or symbioses. Our definitions differ slightly from accepted usage, in which claustral founding refers to queens that are isolated in the incipient nest, regardless of whether they feed. We emphasize, however, the energetic demands of reproduction and therefore classify colony founding as subsidized or not by an outside source. The difference affects one species (Atta colombica, see below), and our overall results were unaffected by its classification. Members of the subfamily Ponerinae (Hypoponera, Odontomachus, Pachycondyla) lack adult storage proteins, which allow queens to store the amino acids necessary for producing workers, and must forage during the founding period, making them non-claustral (Peeters and Ito 2001; Brown and Bonhoeffer 2003). Among the Formicoid clade (in this study, subfamilies Dolichoderinae, Formicinae and Myrmicinae) claustral founding is the predominant reproductive strategy (Hölldobler and Wilson 1990; Brown and Bonhoeffer 2003; Keller et al. 2014). Members of these subfamilies were conservatively assumed to be claustral unless known otherwise (Dolichoderinae—Azteca, Dolichoderus bispinosus, D. lutosus; Formicinae—Brachymyrmex, Camponotus; Myrmicinae—Cephalotes, Crematogaster, Pheidole, Solenopsis, Xenomyrmex), a method that is likely to overlook cases of non-claustral founding as reproductive strategy is unknown for many species. Species known or suspected to found colonies in nests of other social insects (Dolichoderus debilis, D. lamellosus, D. laminatus, AntWeb 2013; Megalomyrmex, Adams et al. 2013) were assumed to have access to food during the founding period and designated non-claustral founders. Leaf-cutters (Atta) found colonies alone but feed on symbiotic fungi during the founding period (Mintzer 1987; Seal 2009; Augustin et al. 2011) and are here designated non-claustral.

Scaling relationships

To characterize how flight morphology varies with body size across the ants, we examined the scaling relationships of flight morphology against body mass using species means (Table 1). We calculated scaling exponents as the slopes of log-log regressions of flight characters against body mass and checked for deviations from isometry by comparing the observed scaling exponents with those predicted by isometric growth. In effect, this examines whether large ants are the same shape as small ants. To account for evolutionary history we repeated the process using phylogenetically independent contrasts of genus averages. One log-transformed variable—wingspan—deviated slightly from normality. The drag-mass relationship showed heteroscedasticity, decreasing in variance with increasing body size, probably as a result of measurement error of small abdomens.

Flight characterization

We characterized the overall flight morphology of species by plotting each species average on a two dimensional “flight ability space.” The axes of the flight ability space represent variation in the two characters most important for predicting insect flight ability—FMR and wing loading. Variation in these traits corresponds predictably to variation in several basic flight parameters—maneuverability, maximum flight time and minimum speed required for flight. FMR values are shown as standard normal values. Wing loading measures are the residuals of a log-log regression of wing loading on body mass. The plot therefore captures variation in wing loading after accounting for effects of body size variation. FMR was independent of body size, so we tested the prediction that claustral founders have lower FMRs than non-claustral founders with a t-test. We compared other traits with ANCOVAs on ln-transformed characters with ln head width as a covariate.

After analysis, two presumed claustral founders—Camponotus nitidior and Brachymyrmex BCILT1—clustered with non-claustral founders in flight ability space, suggesting non-claustral founding. The colony founding strategies of these species are unknown and they were conservatively presumed claustral. To further test whether they are actually non-claustral, we estimated the fat content of these species by calculating the abdomen mass ratio. Fat content is an indicator of reproductive strategy in ants, with fat in claustral founders making up over 40% of body mass (Keller and Passera

1989). Abdomen mass ratio is an overestimate of fat content and therefore a conservative measure in this case.

Data analysis

All statistics were done in R (R Core Team 2012). Variables were checked for normality with the Shapiro-Wilk test. Allometric scaling regressions of species data were standardized major axis regressions using the “lmodel2” package (Legendre 2011) to account for measurement error of independent variables (McArdle 1988). Regressions for calculating residuals used OLS regressions which are more appropriate for prediction (Sokal and Rohlf 1995). Regressions were tested for heteroscedasticity using the “car” package (Fox and Weisberg 2011). For paired comparisons we checked for homogeneity of variance using Bartlett’s test.

For phylogenetically independent comparisons we constructed a genus level tree with the “ape” package (Paradis et al. 2004), using data for tree topology and branch lengths from Moreau et al. (2006). Phylogenetically independent contrasts were done with the “geiger” package (Harmon et al. 2008). Phylogenetically independent scaling regressions of genus averages used OLS regression.

Results

We characterized the flight morphology of 178 queens from 21 species in all four major subfamilies (Table 1). The species spanned four orders of magnitude in body mass with commensurate variation in flight morphology, capturing nearly the entire natural range of variation among flying queens. The largest queens (*Atta colombica*) were nearly 700 times heavier than the smallest (*Pheidole christopherseni*), at 195 mg and 0.29 mg dry weight. FMR ranged from 0.13 (*Crematogaster stollii, Solenopsis BCILT3,* *Xenomyrmex stollii*) to 0.34 (*Pachycondyla harpax*), wing loading from 0.35 Nm^{-2} (*Brachymyrmex BCILT1*) to 4.60 Nm^{-2} (*Atta colombica*), and abdomen drag areas from 0.56 mm^{2} (*Hypoponera q6*) to 40.75 mm^{2} (*Atta colombica*). Wing shape, in contrast, was relatively invariant with aspect ratios ranging from 5.89 (*Dolichoderus debilis*) to 7.09 (*Xenomyrmex stollii*).

Table 1. Ant flight morphology

Subfamily	Species	n	Mass mg	HW mm	FMR	WSpan mm	WArea mm^2	WLoad N/m^2	AR	WMD mg/mm^2	$V^{2/3}$ mm^2	Strategy
Dolichoderinae	Azteca instabilis*	73	21.700	2.4	0.20	22.30	79.39	2.67	6.28	0.0041	10.11	C
	Dolichoderus bispinosus	6	18.649	2.4	0.21	21.07	72.21	2.53	6.15	0.0042	9.35	C
	Dolichoderus debilis	6	1.861	1.1	0.20	9.54	15.47	1.18	5.89	0.0021	3.04	N
	Dolichoderus lamellosus	4	1.424	1.1	0.23	8.05	10.88	1.28	6.07	0.0015	1.38	N
	Dolichoderus laminatus	3	1.338	1.1	0.22	8.17	10.55	1.22	6.31	0.0014	1.84	N
	Dolichoderus lutosus	6	1.606	1.2	0.21	9.33	13.48	1.17	6.47	0.0018	1.82	C
Formicinae	Brachymyrmex BCILT1	6	0.333	0.8	0.29	7.77	9.64	0.35	6.28	0.0013	1.44	C
	Camponotus abdominalis	4	60.161	3.6	0.15	29.43	137.81	3.23	6.29	0.0049	18.01	C
	Camponotus mucronatus	6	2.799	1.1	0.14	12.27	23.15	1.20	6.43	0.0020	3.04	C
	Camponotus nitidior	9	5.950	1.7	0.25	15.19	35.58	1.64	6.50	0.0026	3.61	C
	Camponotus sp1	2	35.552	3.4	0.18	26.14	110.14	3.17	6.21	0.0047	14.18	C
Myrmicinae	Atta colombica	3	193.668	4.8	0.27	52.87	415.27	4.60	6.74	0.0096	40.75	N
	Cephalotes porrasi	2	3.978	1.7	0.22	12.33	24.96	1.56	6.09	0.0021	2.46	C
	Crematogaster stollii	6	19.335	2.3	0.13	21.53	76.40	2.48	6.07	0.0042	10.62	C
	Megalomyrmex symmetochus	6	0.590	0.9	0.30	7.60	9.17	0.63	6.31	0.0017	1.08	N
	Pheidole christopherseni	6	0.285	0.5	0.19	6.80	6.86	0.39	6.70	0.0011	0.58	C
	Solenopsis BCILT3	6	0.461	0.6	0.13	7.90	8.95	0.51	7.01	0.0018	0.90	C
	Xenomyrmex stollii	6	0.602	0.7	0.13	7.33	7.59	0.78	7.09	0.0016	1.23	C
Ponerinae	Hypoponera q6	6	0.457	0.8	0.28	6.70	6.98	0.64	6.44	0.0014	0.56	N
	Odontomachus bauri	6	9.612	2.4	0.25	16.04	40.14	2.34	6.42	0.0030	4.77	N
	Pachycondyla harpax	6	7.191	1.9	0.34	14.97	34.30	2.05	6.54	0.0030	4.24	N

Mass = body dry mass, HW = head width, FMR = flight muscle ratio, WSpan = wingspan, WArea = wing area, WLoad = wing loading, AR = aspect ratio, WMD = wing mass density, $V^{2/3}$ = abdomen drag reference area, C = claustral founding, N = non-claustral founding

Values are species means

*Some data for Azteca instabilis previously published in Helms & Kaspari (2014)

Dimensionless flight morphology metrics—FMR and aspect ratio—were unrelated to body mass. All other characters increased with size, with body mass accounting for most of the variation (Table 2). Wing loading was the only character to deviate from isometry using unadjusted species averages. After accounting for phylogeny and using genus averages, however, all dimensional flight morphology metrics except drag were allometric. With the exception of wing mass density—a measure of wing durability not clearly linked to flight—all deviations from isometry are consistent with decreased relative flight ability in larger ants. Larger species have higher wing loading and shorter and smaller wings than expected from isometric scaling with body mass.

Table 2. Allometric scaling of ant flight morphology with body mass

Character	Predicted exponent	Observed exponent	r^2	PIC exp	r^2	Flight
FMR	0	0	0.003	0	0.01	—
Wing loading	0.333	**0.390**	0.93	**0.404**	0.97	Worse
Drag	0.666	0.629	0.96	0.614	0.97	Better
Aspect ratio	0	0	0.044	0	0.1	—
WMD	0.333	0.302	0.95	**0.288**	0.96	?
Wingspan	0.333	0.309	0.97	**0.289**	0.96	Worse
Wing area	0.666	0.621	0.97	**0.588**	0.98	Worse

Scaling exponents are calculated according to the formula ln (character) = constant + exponent * ln (body mass), by using SMA regressions. Predicted exponents are those predicted by isometric growth. PIC exponents are calculated using phylogenetically independent contrasts with OLS regressions on genus averages. Exponents in bold deviate from isometry at $P < 0.05$. "Flight" shows whether deviations act in a direction to increase or decrease relative flight ability.

The plot of size-corrected wing loading against FMR suggests a phylogenetic component to flight morphology, although sample sizes do not permit high power comparisons between subfamilies (Figure 3). Myrmicines, the most taxonomically diverse subfamily, likewise display the most flight morphological diversity and are widely scattered across the space. Dolichoderines cluster around average FMR and high wing loading, suggesting short duration, high speed, low maneuverability flight relative to other ants. Ponerines are concentrated in the top right quadrant, displaying both high wing loading and high FMR, suggesting moderate to long duration, high speed, moderately to highly maneuverable flight. With the exception of *Brachymyrmex*, Formicines appear to vary primarily in FMR, hovering around average wing loading.

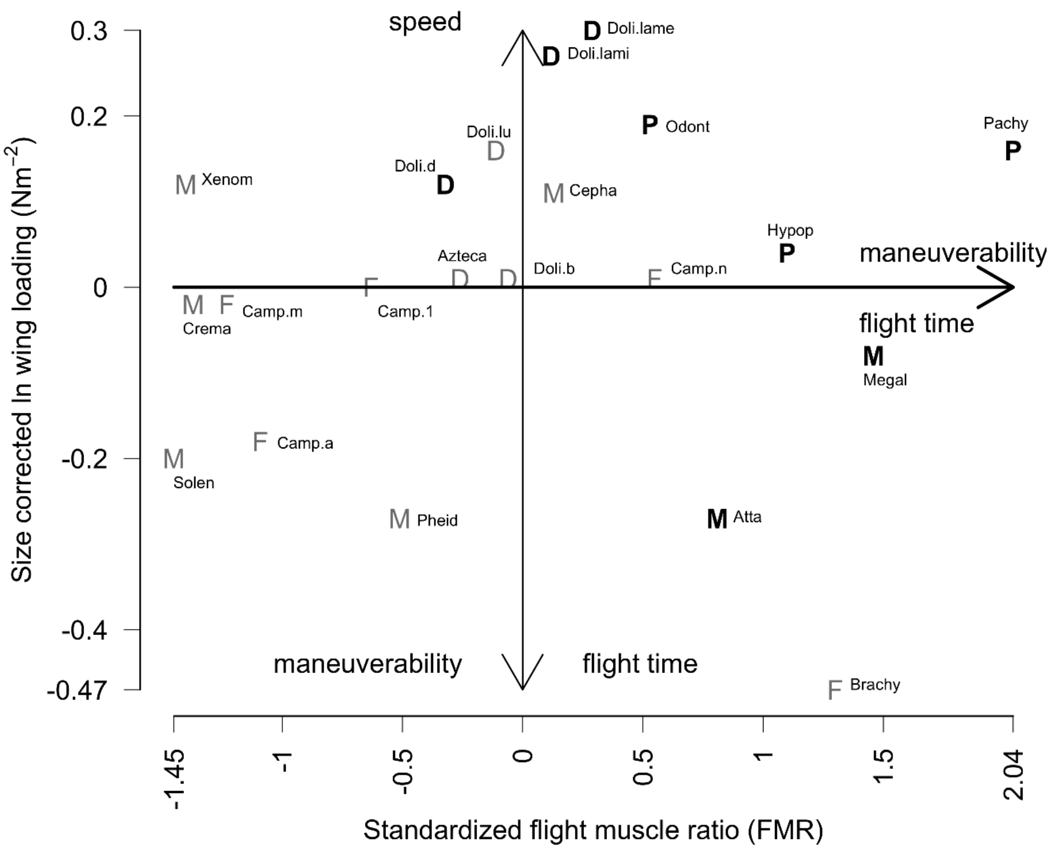

Figure 3. Ant species in flight ability space. Y-axis values are residuals of a log-log regression of wing loading versus body mass. Letters (D, F, M, P) denote subfamilies. Plain text letters represent claustral species and bolded letters those that feed while founding. Arrows and text show how flight performance is predicted to vary, and arrow thickness represents relative importance of each axis. Maneuverability and flight time increase with flight muscle ratio and decrease with wing loading, and minimum flight speed increases with wing loading.

As predicted by FoF (P1), non-claustral founders—located on the right of the flight ability space (Figure 3)—have 40% higher FMRs than claustral founders (0.187 ± 0.05 vs. 0.261 ±0.05, t-test, *P* = 0.003, Figure 4). Also as predicted (P3), claustral founders trended toward larger abdomens with higher abdomen drag (ANCOVA F (1, 18) = 3.860, *P* = 0.065, Tables 3 and 4, Figure 5). Contrary to predictions based only on the role of flight muscle histolysis in colony growth (e.g. Hölldobler and Wilson 1990; Peeters and Ito 2001), we found no difference between claustral and non-claustral founders in size-corrected thorax mass (ANCOVA F (1, 18) 0.0987, *P* = 0.76, Tables 3 and 4, Figure 5).

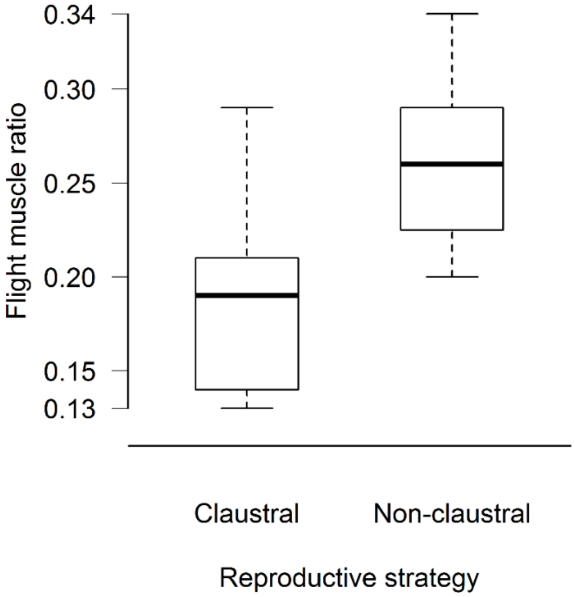

Figure 4. Claustral queens have lower flight muscle ratios than non-claustral species. Boxplots show medians and quartiles of species averages.

Table 3. ANCOVA results testing for flight morphology differences between claustral and non-claustral queens. All variables are ln transformed.

Trait	Factor	df	F	*P*
Wing loading	Head width	1	185.33	<0.001
	Strategy	1	0.100	0.76
	Error	18		
Wing area	Head width	1	338.42	<0.001
	Strategy	1	6.94	0.017
	HW*Strategy	1	6.38	0.022
	Error	17		
Drag	Head width	1	216.90	<0.001
	Strategy	1	3.86	0.065
	Error	18		
Thorax mass	Head width	1	887.37	<0.001
	Strategy	1	0.099	0.76
	Error	18		

Table 4. Regressions of flight morphology on head width. All variables are ln transformed.

Character	Strategy	Slope	Intercept	r^2
Wing loading	C	1.13	-0.13	0.90
	N	1.05	-0.66	0.93
Wing area	C	1.60	2.79	0.95
	N	2.11	2.27	0.96
Drag	C	1.67	0.66*	0.94
	N	2.01	0.23*	0.91
Thorax mass	C	2.82	-1.35	0.98
	N	3.19	-1.45	0.99

Pairs in bold differ at $P < 0.05$

* denotes marginally significant difference at $P = 0.065$

Contrary to prediction (P2), species appeared to break the wing loading tradeoff through evolutionary events. Despite higher nutrient loads in claustral founders, the two groups did not differ in size-corrected wing loading (ANCOVA F (1, 18) = 0.0998, *P* = 0.76, Tables 3 and 4). Instead, claustral founders had larger wings than non-claustral founders (ANCOVA F (1, 17) = 6.938, *P* = 0.017, Tables 3 and 4, Figure 5), compensating for wing loading effects of heavier abdomens.

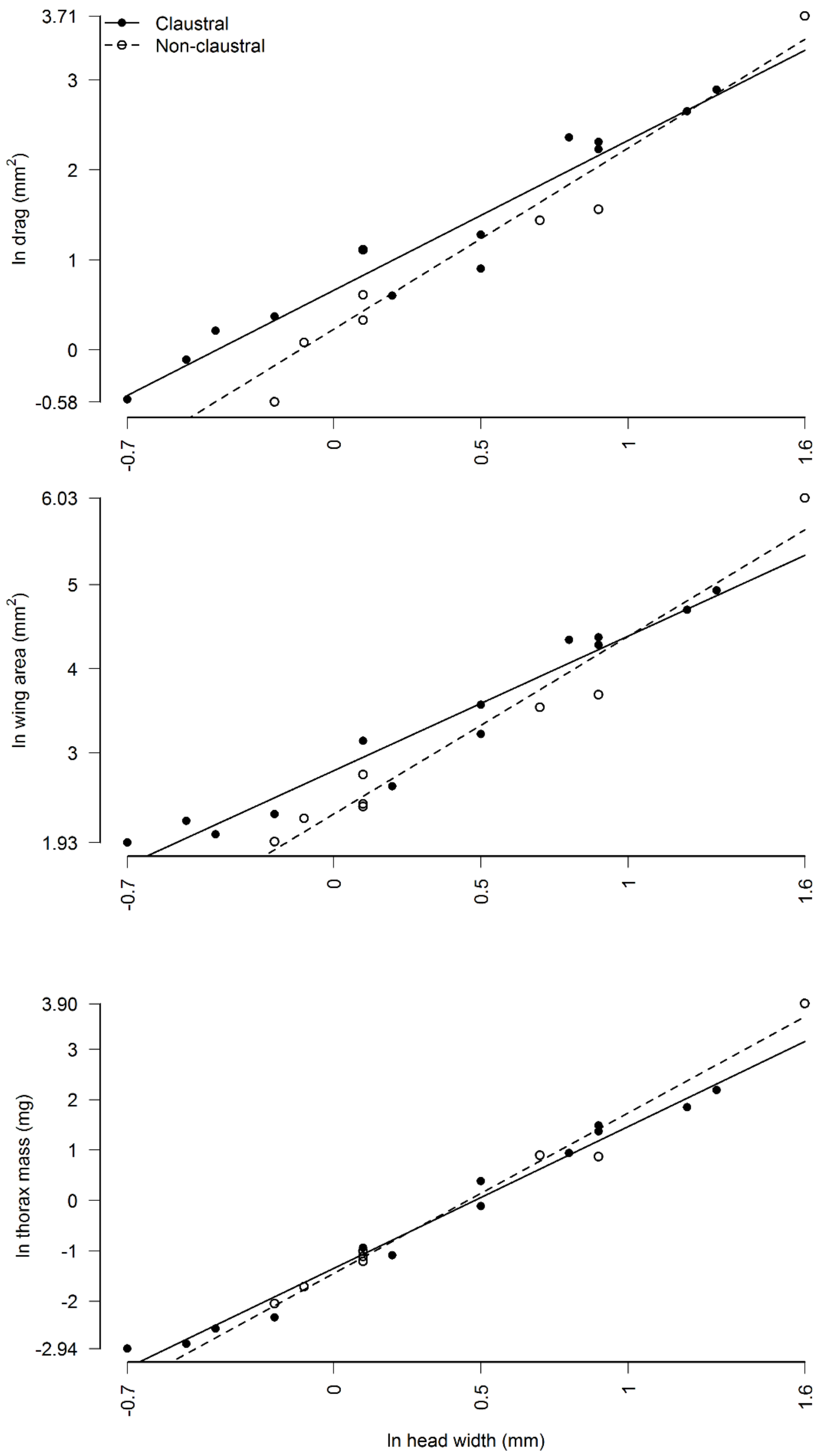
Claustral
Non-claustral
ln drag (mm^2)
3.71
3
2
1
0
-0.58
-0.7
0
0.5
1
1.6
ln wing area (mm^2)
6.03
5
4
3
1.93
-0.7
0
0.5
1
1.6
ln thorax mass (mg)
3.90
3
2
1
0
-1
-2
-2.94
-0.7
0
0.5
1
1.6
ln head width (mm)

Figure 5. Abdomen drag, wing area and thorax mass versus body size in claustral and non-claustral queens. Claustral queens trend toward higher drag as a result of larger abdomens. They also have larger wings which offset potential wing loading increases from heavier abdomens. Claustral queens do not have heavier thoraces.

Although we conservatively treated them as claustral in all our analyses (see Methods), the high FMRs of *Camponotus nitidior* (0.25 ±0.02) and *Brachymyrmex BCILT1* (0.29 ±0.04) were similar to non-claustral species. Their abdomen mass ratios (*C. nitidior*, 0.25 ±0.05; *B. BCILT1*, 0.32 ±0.07) also suggested non-claustral founding. All other presumed claustral founders in this study were over 40% abdomen mass.

FMRs of five species—all claustral founders (P4)—were in the marginal range where insects lose the ability to fly—0.12 to 0.16 (Table 1, Figure 6). Four species—*Camponotus mucronatus*, *Crematogaster stollii*, *Solenopsis BCILT3*, and *Xenomyrmex stollii*—had queens at 0.12 or below, with a low of 0.11 in two individuals of *S. BCILT3*. Moreover, all our queens were captured in flight and we overestimated FMR (see Methods), so the marginal FMR for these species is probably less than 0.13 and for some is likely below 0.11. These queens therefore had among the lowest FMRs of any flying insect.

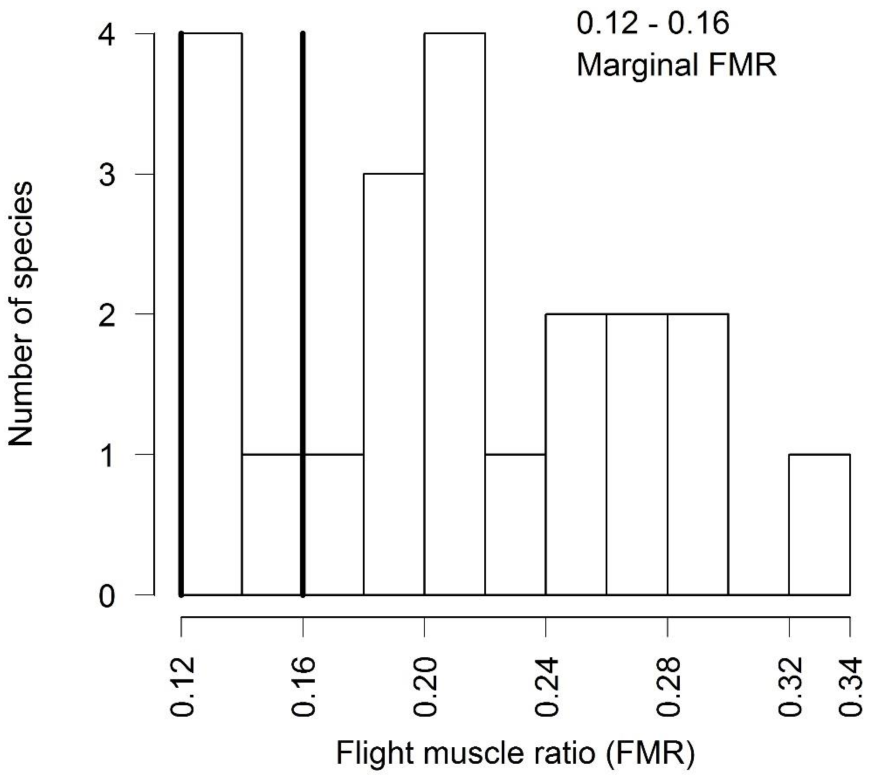

Figure 6. Ant flight muscle ratios. Dark lines denote marginal FMRs which mark the threshold of flightlessness in other insects. All five species within this range are claustral founders.

Discussion

Flight is central to ant life histories. FoF emphasizes the link between flight and reproduction to predict how flight shapes queen evolution. Consistent with FoF, our results suggest the evolution of claustral founding reduces dispersal ability by (P1) lowering FMR and (P3) increasing abdomen drag. Claustral founders are expected to be less maneuverable and fly at narrower temperature ranges and for shorter periods than non-claustral queens of similar size, reducing their ability to evade predators, find suitable nest sites and disperse long distances. On the other hand, wing loading (P2) was invariant with strategy because of evolutionary changes in wing area. Several claustral species had FMRs on the verge of flightlessness (P4) and crossing this threshold is a possible mechanism for recurrent loss of flight in ant lineages. These relationships support the view that a queen's morphology reflects the conflicting demands of flight and post-dispersal survival. This is reminiscent of tradeoffs in plants and other insects (Harrison 1980; Guries and Nordheim 1984; Wagner and Liebherr 1992; Zera and Denno 1997; Marden 2000), suggesting that ants model a general reproduction-dispersal tradeoff.

We focus on females because queens—incipient colonies—are the relevant dispersal units for populations. Gene flow, however, is mediated by both sexes and studies of male flight are necessary to fully explore genetic consequences of dispersal differences (Peeters 2012). Males likely experience dispersal tradeoffs related to sperm load and mating behavior (Davidson 1982; Fjerdingstad & Boomsma 1997; Shik et al. 2013), and their morphology may covary with that of queens. Comparing population structures of claustral and non-claustral founders, while controlling for effects of varying queen number (Seppä et al. 1995; Liautard & Keller 2001; Hannonen et al. 2004), would further test FoF and illuminate the gene flow role of males.

Emphasis on the metabolic role of flight muscles in fueling colony growth, as opposed to their primary flight function, has led to the prediction that claustral founders have larger flight muscles than non-claustral founders (e.g. Hölldobler and Wilson 1990; Peeters and Ito 2001). Flight muscle, however, plays a secondary role in fueling colony growth and abdominal reserves are the primary source of energy and amino acids in founding queens (Wheeler and Buck 1995, 1996; Wheeler and Martinez 1995; Brown and Bonhoeffer 2003). We find flight muscles in claustral founders are actually smaller relative to body mass, as predicted by FoF, and find no difference in absolute thorax mass (Keller and Ross 1993). If claustral founders do evolve larger flight muscles, it would likely be in response to the increased abdominal weight to be carried (Peeters and Ito 2001) rather than as an energy source.

Several claustral species flew at FMRs impossible for other insects, suggesting unknown adaptations for load-bearing flight. We captured queens flying with FMRs of 0.11, carrying what may be the heaviest body load documented in flying insects (Marden 1987, 2000). The closest relatives of ants—bees and wasps—lose the ability to fly at FMRs below 0.18 (Marden 1987). We speculate that this superior load-bearing ability is a response to the selective demands of nutrient loading for claustral founding (Keller and Passera 1989). Further, by capturing queens ranging from high FMR to these marginal values we begin to address the evolution of flightless queens. Researchers view flightlessness in ants as the result of a qualitative tradeoff between nutrient loading and dispersal (e.g. Sundström 1995; Heinze and Keller 2000). By recognizing quantitative variation within flying queens rather than lumping ants as flying or flightless, we extend the tradeoff to all ants and illustrate a potential mechanism for the evolution of flightlessness. Because extreme nutrient loads would cause queens to drop below marginal FMR, flightlessness may evolve automatically in response to selection for greater nutrient loads in founders, provided they have an alternate dispersal method. Further changes associated with flightlessness, such as shortening or loss of wings (Heinze and Keller 2000) or reductions in flight muscle

(Peeters et al. 2012), may follow. This simple mechanism may explain the ubiquity of flightlessness, which occurs in over 50 genera from all major subfamilies of ants (Peeters 2012).

Finally, flight morphology may predict life history (Keller et al. 2014). Two presumed claustral founders—*Camponotus nitidior* and *Brachymyrmex BCILT1*—had flight muscle and abdomen mass ratios similar to non-claustral species. Claustral founding is the rule in cavity-dwelling *Camponotus*. *Camponotus nitidior*, however, founds colonies on leaf surfaces (AntWeb 2013), making queen foraging possible. In support of this idea, *C. nitidior* queens also appear to have well developed worker-like neck muscles, an indicator of queen foraging (Keller et al. 2014). Almost nothing is known about colony founding in *Brachymyrmex*, but many species are mutualists of plant-feeding hemipterans (AntWeb 2013). Queens may found colonies among symbionts and thereby obtain food. Although pure speculation, either situation—queen foraging or symbiosis—would mean the species are actually non-claustral as suggested by flight morphology.

Flight links the primary functions of ant queens—dispersal and reproduction—and thereby plays a fundamental role in their ecology. We illustrate several examples in which flight shapes queen biology. Likewise, examination of flight should grant insight into dispersal polymorphisms (Bourke and Franks 1991; Keller and Ross 1993; Sundström 1995) and invasions (Markin et al. 1971; Yamauchi and Ogata 1995; Rüppell and Heinze 1999). By recognizing the coupling of reproduction and flight, and highlighting reproduction-dispersal tradeoffs, FoF provides a useful starting point for addressing these issues and others. Ants are flyers and to understand them we must expand our focus from what they do on the ground to ask what they do in the air.

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Chapter 3. Dispersal polymorphisms in invasive fire ants

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Abstract

In the Found or Fly (FoF) hypothesis ant queens experience reproduction-dispersal tradeoffs such that queens with heavier abdomens are better at founding colonies but are worse flyers. We tested predictions of FoF in two globally invasive fire ants, *Solenopsis geminata* (FABRICIUS, 1804) and *S. invicta* (BUREN, 1972). Colonies of these species may produce two different monogyne queen types—claustral queens with heavy abdomens that found colonies independently, and parasitic queens with small abdomens that take over conspecific nests. Claustral and parasitic queens were similarly sized, but

the abdomens of claustral queens weighed twice as much as those of their parasitic counterparts. Their heavier abdomens adversely impacted morphological predictors of flight ability, resulting in 32-38% lower flight muscle ratios, 55-63% higher wing loading, and 32-33% higher abdomen drag. In lab experiments maximum flight durations in claustral *S. invicta* queens decreased by about 18 minutes for every milligram of abdomen mass. Combining our results into a simple fitness tradeoff model, we calculated that an average parasitic *S. invicta* queen could produce only 1/3 as many worker offspring as a claustral queen, but could fly 4 times as long and have a 17- to 36-fold larger potential colonization area. Investigations of dispersal polymorphisms and their associated tradeoffs promises to shed light on range expansions in invasive species, the evolution of alternative reproductive strategies, and the selective forces driving the recurrent evolution of parasitism in ants.

Introduction

Life history tradeoffs between dispersal and reproductive or competitive ability are known for many organisms (Diamond 1974; Werner & Platt 1976), including insects (Harrison 1980; Wagner & Liebherr 1992; Zera & Denno 1997). Here we examine one such example in ant queens, in which the Found or Fly hypothesis (FoF) posits a tradeoff between colony founding and flight ability mediated by abdominal nutrient loads (Helms & Kaspari 2014). In most species young queens fly from their natal nests to mate and disperse (Hölldobler & Wilson 1990; Peeters & Ito 2001). After finding a suitable nest site a queen sheds her wings, lays eggs and grows a new colony (Tschinkel 1988c). Heavier abdomens, containing more fat and protein reserves (Peakin 1972; Keller & Passera 1989; Martinez & Wheeler 1994), increase a founding queen's survival and reproductive output (Mintzer 1987; Bernasconi & Keller 1999; DeHeer 2002). At the same time, heavier nutrient loads negatively impact flight morphology by decreasing flight muscle ratio (FMR) and increasing wing loading and abdomen drag (Helms & Kaspari 2014), changes which likely hinder a queen's ability to disperse long distances or search for nest sites (Rüppell et al. 1998; Lachaud et al. 1999).

This tradeoff is both ecological, playing out during an individual queen's lifetime as she gains abdominal weight (Helms & Kaspari 2014), and evolutionary, causing species with different reproductive strategies to differ also in flight morphology (Helms & Kaspari 2015). The evolution of the most common strategy, claustral founding, in which founding queens are isolated and survive entirely off their abdominal reserves (Keller & Passera 1989; Brown & Bonhoeffer 2003; Keller et al. 2014), likely incurs a cost in dispersal ability (Helms & Kaspari 2015). At the opposite extreme, socially parasitic species found colonies inside the nests of other ants and manipulate the native workers into raising foreign offspring (Buschinger 1986, 2009). Because parasitic queens take over fully functioning colonies, complete with food reserves and foraging workers, they store no abdominal nutrients and are unable to found colonies independently (Keller & Passera 1989) but may be better dispersers. Parasitism is common among ants, with over 200 known parasitic species arising from dozens of independent origins (Buschinger 1986, 2009), and parasites may constitute up to a third of the ant species in some regions (Buschinger 1986). Most parasites are sister species to their hosts, or at least closely related (Emory 1909), and parasite-host pairs likely evolve sympatrically from an ancestral host-like species (West-Eberhard 1986, 2005; Bourke & Franks 1991; Buschinger 2009). Indeed, many species are facultatively parasitic, capable of producing two different queen types within the same colony and from the same genome (Bourke & Franks 1991; Rüppell & Heinze 1999). In these queen polymorphic species, one queen type founds colonies in the typical claustral manner and the other founds colonies parasitically by taking over conspecific nests. The two queen types often fly and mate at different times of the year and may also differ in size or morphology (Buschinger 1986, 2009; Bourke & Franks 1991). In the simplest cases the queen types differ only in abdomen weight (Tschinkel 1996). Queen polymorphic ant species are thus ideal systems for studying reproduction-dispersal tradeoffs, as they allow us to isolate the effects of reproductive strategy and nutrient loading while controlling for evolutionary history, ecology, geography and even genetic variation.

Differences in size, morphology, or mating season among polymorphic queens likely lead to differences in dispersal ability (Ross & Keller 1995; Sundström 1995; Heinze & Keller 2000). Except in cases where queens differ in gross wing morphology (Heinze & Tsuji 1995; Heinze & Keller 2000), however, evidence for dispersal polymorphisms in parasitic species is lacking. Here we examine two queen polymorphic fire ant species that co-occur (Tschinkel 1988b) in the southeastern United States—the tropical fire ant (*Solenopsis geminata* FABRICIUS, 1804) and the red imported fire ant (*S. invicta* BUREN, 1972) (Figure 7). Dispersal studies are particularly relevant in these cases because both fire ants are global invaders whose non-native ranges are currently expanding through active dispersal during mating flights (Wetterer 2011; Tschinkel 2013a; Gotzek et al. 2015). The two species are facultative parasites, with colonies producing both claustral and parasitic queens that differ primarily in abdomen weight and fly at different times of the year (McInnes & Tschinkel 1995; Tschinkel 1996). Colonies produce claustral queens during the spring and summer to found new colonies independently. The lighter parasitic queens, in contrast, fly and mate in the fall (*S. geminata*) or late winter (*S. invicta*), enter conspecific colonies whose queens happen to have died during the year, and manipulate the orphaned workers into adopting them as their new queen.

The two species differ, however, in how queens become claustral or parasitic. In *S. geminata* the two queen types experience slightly different developmental trajectories (McInnes & Tschinkel 1995), allowing their morphologies to diverge in response to dispersal tradeoffs. For example, claustral queen types might compensate for their heavier abdomens, as claustral species do in interspecific comparisons, by developing larger wings than parasitic queens (Helms & Kaspari 2015). In *S. invicta*, in contrast, queen type appears not to be developmentally determined, as the queens differ only in adult weight gain and behavior (Tschinkel 1996; DeHeer & Tschinkel 1998). Queen types in *S. invicta* therefore represent alternate ways to use the same underlying body, precluding evolutionary alterations to one queen type's morphology independently of the other. The similarities between the species thus provide two replicate study systems, while their differences allow us to explore the dispersal consequences of different modes of caste determination.

Figure 7. Queens of Solenopsis geminata (A, B) and S. invicta (C, D). In both species claustral queens (A, C) store more nutrients and have larger abdomens than parasitic queens (B, D) (Photos by Brittany Benson).

We test for dispersal polymorphisms in these species in the context of the Found or Fly hypothesis using a three-pronged approach—we first test our assumptions, then we test for flight morphology differences among queen types, and finally we link flight morphology to flight performance. FoF assumes A1) that queen types differ in abdomen mass due to the different energetic loads required for reproduction, and A2) that heavier abdomens adversely impact flight morphology. Because of the abdomen mass differences, FoF predicts that claustral queens will have P1) lower flight muscle ratios, P2) higher wing loading, and P3) higher abdomen drag. Insect wings are evolutionarily labile (Mezey & Houle 2005), however, and populations may break the wing loading tradeoff by evolving larger wings (Helms & Kaspari 2015). We therefore predict that in *S. geminata*, in which queen types differ developmentally, P4) claustral queens will develop larger wings to compensate for higher abdomen loads. In contrast, in *S. invicta*, in which queen types experience the same developmental program, P5) queen morphs will not differ in wing size. Translating these morphological differences into dispersal ability, we predict queens with heavier abdomens will P6) have shorter flight durations. We test these predictions by studying the morphology and flight behavior of naturally varying queens. Finally, we combine our results in a simple model that links reproductive strategy, abdomen mass, reproductive output, and flight ability.

Materials and methods

Locality and specimens

All specimens were collected and all experiments performed in 2013 in and around Tallahassee, northern Florida, USA (30°27’18’’N 84°15’12’’W). Both target fire ant species co-occur here (Tschinkel 1988b), *S. geminata* as a native or ancient invasive species (Trager 1991) and *S. invicta* as a recent invasive (Tschinkel 2013a). Studied populations of both species were monogyne (having one queen per colony), although polygyne *S. geminata* have been collected in Florida (Adams et al. 1976; Trager 1991) and polygyne *S. invicta* occur in the Tallahassee area at low frequencies (King et al. 2008). No permits were required to sample the ants, as they were hand collected along public roadsides, and no protected or endangered species were involved.

Flight morphology

To test morphological assumptions and predictions we collected virgin queens that had not yet flown, either from on top of their nests as they left for their mating flights or by excavating them from the upper layers of mature nests during the mating season, and preserved them in ethanol. We collected parasitic *S. invicta* in late winter (13 to 17 March), claustral *S. invicta* and claustral *S. geminata* in late spring and summer (18 to 25 June and 18 June to 12 July), and parasitic *S. geminata* in autumn (20 to 21 November). We collected *S. geminata* queens from sandy soils in longleaf pine (*Pinus palustris*) savannas within Apalachicola National Forest, and *S. invicta* queens from lawns and roadsides in the Tallahassee area. For each queen type we sampled 13 to 58 individuals representing three to six separate colonies and measured their flight morphology. Differences among queen castes in these species are comparable to those among heterospecific queens, which can be detected with sample sizes as low as three to six (Helms & Kaspari 2015). Pilot studies showed that variance in fire ant flight morphology measurements stabilized after about seven individuals (mean 6.9 ±4.7, n = 16 morphology by queen type measurements).

We processed each specimen according to a protocol adapted from (Helms & Kaspari 2014). For the sake of clarity we refer to the mesosoma as the *thorax* and the gaster as the *abdomen.* To measure body and abdomen size we measured head width, abdomen length and abdomen height to 0.1 mm with an ocular micrometer under a dissecting microscope. Head width—the maximum width of the head in full-face view excluding the eyes—is a standard measure of ant body size. Abdomen length is the maximum length of the abdomen measured from the dorsal point of attachment of the post-petiole. Abdomen height is the maximum height of the abdomen in profile view. After linear measurements we separated the abdomen, thorax, wings and other body parts and dried them for 48 to 72 hrs at 60 to 65 °C. We weighed the dried abdomen, thorax, wings and entire body to 0.001 mg using a Cahn microbalance. After weighing we placed one forewing and one hindwing from each specimen onto a slide and photographed them with a reference ruler under a Leica dissecting microscope camera. We then measured wing lengths and wing areas using ImageJ software (Schneider et al. 2012).

After processing the specimens we calculated flight morphology metrics for each queen. Flight muscle ratio (FMR)—the ratio of flight muscle mass to body mass—is probably the most important predictor of insect flight performance (Marden 1987, 2000; Ellington 1991; Dudley 2000). FMR is proportional to acceleration and load lifting ability, and a higher FMR increases maneuverability, flight endurance and the temperature range at which an insect can fly. We calculated FMR by dividing the thorax mass by total body mass. Thorax mass is a standard surrogate for flight muscle in ants and other insects (Fjerdingstad & Boomsma 1997; Norberg & Leimar 2002; Dillon & Dudley 2004). To ensure queen types did not differ in flight muscle development we dissected a voucher specimen of each type to look for atrophied or absent flight muscle. In all cases flight muscle was well developed and filled the thorax, justifying the use of thorax mass as a surrogate for flight muscle mass. Another metric, wing loading—the ratio of body weight to wing area—decreases maneuverability, flight endurance and maximum flight speed, and increases minimum power and speed requirements for flight (Norberg & Rayner 1987; Hedenström 1992;

Vogt et al. 2002; Darveau et al. 2005; Srygley & Dudley 2008). We calculated wing loading by dividing body mass by the combined area of all four wings (mg/mm^2). A third metric, abdomen drag, increases the power requirements of flight and reduces overall flight performance (Ellington 1991; Dudley 2000). Drag is determined by an object's size and shape and is proportional to a two-dimensional reference area. We use a volumetric reference area—abdomen volume$^{2/3}$ (mm^2)—which links mass to size and shape (Alexander 1990; Vogel 1994). We calculated abdomen volume using the formula for a prolate spheroid, using abdomen length and height as the major and minor axes. Finally, we calculated two aspects of wing morphology that are independent of abdomen mass and hence not likely to vary with reproductive strategy. Aspect ratio—wing narrowness—equals 4*forewing length^2/total wing area. Narrower wings—higher aspect ratios—increase aerodynamic efficiency (Norberg & Rayner 1987; Rayner 1988; Dudley 2000). Wing mass density—a measure of stiffness and durability—is total wing mass divided by total wing area (mg/mm^2).

To control for body size differences when comparing flight morphology we first checked whether parasitic and claustral queens differed in head width. We then tested whether parasitic queens had lighter abdomens than claustral queens (A1). To examine how abdomen mass impacts flight morphology (A2) we regressed flight muscle ratio, wing loading and drag against abdomen mass separately for each queen type. We then tested flight morphology predictions (P1 to P5) by comparing queen types (Table 5).

Table 5. Flight morphology terms and predictions

Trait	Definition	Predictions
Flight muscle ratio (FMR)	Ratio of flight muscle mass to body mass. Increases maneuverability, acceleration, load lifting ability, and the ability to fly at cooler temperatures.	Lower in claustral queens
Wing loading (mg/mm^2)	Ratio of body mass to wing area. Decreases maneuverability, endurance, and maximum flight speed. Increases power and speed requirements.	Higher in claustral queens
Abdomen drag (mm^2)	Theoretical area proportional to the drag experienced in flight. Increases power requirements and reduces performance.	Higher in claustral queens
Wing area (mm^2)	Area of all four wings	*S. geminata* Higher in claustral queens *S. invicta* No difference between types
Forewing length (mm)	Length of the front wings	*S. geminata* Higher in claustral queens *S. invicta* No difference between types
Aspect ratio	Wing narrowness. Increases aerodynamic efficiency.	No difference between types
Wing mass density (mg/mm^2)	Wing stiffness or durability.	No difference between types
Flight duration (s)	The amount of time tethered queens fly during six consecutive take off events.	Decreases with abdomen mass Increases with flight muscle ratio Decreases with wing loading

Live flight

We followed up flight morphology comparisons with a live flight experiment to link morphology to dispersal ability. The experiment was performed from June to July using claustral *S. invicta* queens. We used claustral *S. invicta* queens because they were reliably available in sufficient numbers, perform well in lab conditions, and are routinely used as models in ant biology (Tschinkel 2013a). We collected virgin queens that had not yet flown by excavating them from the top layers of mature colonies in the morning, along with soil and workers from the nest. These colony fragments were kept in plastic containers in the lab and given water. To avoid weight loss or other effects of captivity on flight (Shelton et al. 2006), queens participated in experiments within three days of their collection. Colony fragments remained vigorous and displayed normal behavior throughout this time. Flight experiments were performed in indoor chambers linked to the outside environment through screened windows. Temperature, humidity and barometric pressure thus reflected normal mating season weather conditions but with strong air currents eliminated.

To examine how abdomen mass impacts flight endurance (P6) we observed 33 queens from three colonies during tethered flight (Moser 1967; Davis 1984; Gu & Barker 1995). Flights were performed from 0900 to 1800 at temperatures ranging from 27.0 to 29.6 °C and relative humidity ranging from 67 to 79%, approximating the natural range of flight conditions (Tschinkel 2013a). We tied a 30.5 cm lightweight (0.117 mg/cm) polyester string around the petiole of each queen. About 2.5 cm were used in tying, leaving a 28 cm tether. We clipped the tether to the end of a wooden rod projecting 25 cm horizontally from a table top 75 cm above the ground. We induced queens to fly by gently scraping them off a wooden stick or by blowing on them. Once a queen took off we timed her with a stop watch until she either landed on the rod or stopped flying and hung from the tether. We made each queen fly for six consecutive trials or until she would not take off, and filmed flights with a digital camcorder. For each queen we added all flight durations together to calculate a total flight time. We used total flight time because we are interested in a queen's maximum dispersal performance, but total flight time and average time per bout are tightly correlated (r^2 = 0.98) and the results would be similar for either measure. After the flights we preserved queens in ethanol and processed them as above to compare their performance to their flight morphology. We removed as an outlier one queen with an anomalously light abdomen who had likely just eclosed and was not prepared for her mating flight, leaving 32 queens for analysis.

Factors other than biomechanical considerations likely influence flight duration, such that queens with high potential flight endurance may still fly for only a short time. We thus predicted that queens with heavy abdomens, and thus lower flight muscle ratios and higher wing loading, could have only short flights but those with light abdomens could have long or short flights. In other words, the maximum and range of total flight times should decrease in queens with heavier abdomens (Table 5). Quantile regressions are ideal for characterizing such heterogeneous relationships (Cade & Noon 2003). In our case, we used quantile regressions through the upper quartile to compare maximum values of total flight time to abdomen mass, flight muscle ratio and wing loading. Maximum performance is particularly relevant in ant dispersal studies, as long distance dispersal events impact colony founding success and population occurrence (Bruna et al. 2011), especially during the spread of invasives in novel environments (Tschinkel 2013a). Maximum dispersal ability is of additional applied importance in the case of *S. invicta*, as its rate of spread in the United States (up to 48 km/yr, Hung & Vinson 1978) has exceeded by an order of magnitude estimates of its dispersal ability (<1.6 to 5.4 km, Markin et al. 1971; Vogt et al. 2002), due to long distance dispersal events (Wojcik 1983; Tschinkel 2013a). Despite our primary interest in maximum flight performance, however, we also performed ordinary least squares regressions and nonparametric Spearman's rank correlations as measures of central tendency and for comparison to the upper quartile results.

As an additional measure of flight performance we attempted to measure queen flight distances when dropped. Fifty-six queens were dropped from a height of 170 cm above the center of a 2 x 2 meter chamber, and their resultant flight distances measured. This experiment detected a possible unimodal relationship between abdomen mass and flight distance. The explanatory power, however, was low ($r^2 = 0.13$), likely because of the unrealistic limits of the flight chamber and method of flight initiation (dropping from a height versus taking off from a surface), and we excluded it from our results as uninformative.

Tradeoff model

To further explore the tradeoff between reproduction and dispersal we translated our live flight results to the complete range of parasitic and claustral *S. invicta* abdomen masses by extending the curve derived from our tethered flight experiment. As a measure of reproductive output over the same range we adapted a formula that relates claustral *S. invicta* abdomen mass to the production of first generation workers, based on field and laboratory experiments (Porter & Tschinkel 1986; Tschinkel 1993a). Early workers are reared entirely from queen nutrient reserves, and this relationship describes the number of workers that can be produced from a given abdomen mass. For flight speed comparisons we used a formula that relates tethered *S. invicta* flight speeds to total body mass (Vogt et al. 2002). In constructing this speculative model we make several assumptions. First, in extending the flight endurance curve we assume that flight time, rather than dropping to zero, levels off at about 160 seconds in the heaviest queens, a realistic flight time as *S. invicta* queens often fly less than 400 meters (Tschinkel 2013a). Second, to adapt the worker production curve, which applies to live weight instead of dry weight, we assume a live to dry weight ratio of two (Porter & Tschinkel 1985). Third, we assume that abdomen mass increases consist of fat and protein that is all converted to offspring production. Finally, we assume that patterns derived from claustral queens apply equally to parasitic queens of the same species.

Data analysis

Throughout our analyses we treat individual queens as independent samples. Because we usually measured multiple queens per colony, however, many individuals were sisters whose morphology or flight performance may not have been independent from that of other queens. We dealt with this in several ways. In the case of flight morphology comparisons, we repeated all our analyses using colony averages rather than individual queens, treating each sampled colony as a single data point. When analyzing flight durations, we included colony identity as a factor in all regressions but it was never significant. We also tested whether colonies used in the flight experiments differed in any of the variables analyzed, and found that they did not. We therefore excluded colony identity as a factor in the final flight duration analyses

All statistics were performed in R (R Core Team 2012). We checked normality of variables with the Shapiro-Wilk test. Paired comparisons used *t*-tests for normally distributed variables, presented as means and standard deviations, and Kruskal-Wallis tests for non-normal variables, presented as medians and interquartile ranges (IQR). For the tethered flight experiment, flight muscle ratio was log transformed to meet normality assumptions. Quantile regressions were performed with the *quantreg* R package (Koenker 2013). To account for experimentwise error we applied the Holm-Bonferroni correction (Holm 1979) to *p*-values of regressions of flight morphology versus abdomen mass within queen types, and to regressions of flight duration versus morphology.

Results

Flight morphology

We compared the flight morphology of 142 queens from three mating seasons throughout the year (13 claustral *S. geminata*, 38 parasitic *S. geminata*, 58 claustral *S. invicta*, and 33 parasitic *S. invicta*, Table 6A). Head widths did not differ between queen types in *S. geminata* (Kruskal-Wallis $p = 0.062$, parasite median 1.6 mm, IQR = 1.5–1.6, claustral median 1.6 mm, IQR = 1.6-1.6) or *S. invicta* (Kruskal-Wallis $p = 0.078$, parasite median 1.4 mm, IQR 1.35-1.40, claustral median 1.4 mm, IQR 1.4-1.5). Despite having similar body sizes, the abdomens of claustral *S. geminata* queens (A1) were 2.3 times heavier than those of parasitic queens (5.652 ±0.93 mg versus 2.453 ±0.24 mg, $p = 2.3 \times 10^{-8}$). Likewise, abdomens of claustral *S. invicta* averaged nearly double the weight of their parasitic counterparts (5.331 ±0.91 mg versus 2.745 ±0.67, $p = 2.2 \times 10^{-16}$). We obtained similar results when comparing averages among colonies (Table 6B). Colony average head widths did not differ between queen types in *S. geminata* ($p = 0.77$, parasite mean 1.59 ±0.090 mm, claustral mean 1.58 ±0.071 mm) or *S. invicta* ($p = 0.12$, parasite mean 1.37 ±0.063 mm, claustral mean 1.44 ±0.054 mm), and abdomens of claustral queens were 2.5 times heavier than parasitic queens in *S. geminata* ($p = 0.002$, claustral mean 5.856 ±0.46 mg, parasitic mean 2.342 ±0.24 mg) and 2.1 times greater in *S. invicta* ($p = 7.9 \times 10^{-5}$, claustral mean 5.555 ±0.50 mg, parasitic mean 2.672 ±0.74 mg). Within each queen type heavier abdomens adversely impacted flight morphology (A2) by decreasing flight muscle ratio by 11 to 53%, increasing wing loading by 35 to 122%, and increasing drag by 23 to 95% over their respective ranges of abdomen mass (Figure 8, Table 7).

Table 6. Fire ant queen flight morphology

A. Individual queens

	Solenopsis geminata		*Solenopsis invicta*	
	Claustral	Parasitic	Claustral	Parasitic
n	13	38	58	33
Head width (mm)	1.60 (0.058)	1.56 (0.076)	1.41 (0.075)	1.39 (0.062)
Dry mass (mg)	7.751 (0.95)	4.101 (0.35)	7.242 (0.95)	4.690 (0.75)
Abdomen mass (mg)	5.652 (0.93)	2.453 (0.24)	5.331 (0.91)	2.745 (0.67)
Flight muscle ratio	0.13 (0.018)	0.19 (0.009)	0.15 (0.024)	0.24 (0.035)
Wing loading (mg/mm2)	0.230 (0.029)	0.141 (0.010)	0.268 (0.034)	0.173 (0.028)
Forewing length (mm)	7.19 (0.14)	6.66 (0.10)	6.48 (0.10)	6.57 (0.12)
Total wing area (mm2)	33.7 (1.1)	29.1 (0.9)	27.0 (0.9)	27.6 (1.0)
Aspect ratio	6.14 (0.19)	6.11 (0.10)	6.23 (0.15)	6.24 (0.17)
Wing mass density (mg/mm2)	0.0042 (0.002)	0.0050 (0.0006)	0.0047 (0.001)	0.0059 (0.001)
Abdomen drag (mm2)	4.82 (0.46)	3.66 (0.42)	4.76 (0.60)	3.58 (0.56)

B. Colony averages

	Solenopsis geminata		*Solenopsis invicta*	
	Claustral	Parasitic	Claustral	Parasitic
n	3	5	4	6
Head width (mm)	1.58 (0.071)	1.59 (0.090)	1.44 (0.054)	1.37 (0.063)
Dry mass (mg)	7.957 (0.51)	4.022 (0.36)	7.504 (0.55)	4.642 (0.80)
Abdomen mass (mg)	5.856 (0.46)	2.342 (0.24)	5.555 (0.50)	2.672 (0.74)
Flight muscle ratio	0.13 (0.005)	0.19 (0.006)	0.14 (0.0008)	0.24 (0.039)
Wing loading (mg/mm2)	0.239 (0.018)	0.136 (0.010)	0.274 (0.013)	0.168 (0.034)
Forewing length (mm)	7.12 (0.14)	6.69 (0.11)	6.49 (0.057)	6.58 (0.082)
Total wing area (mm2)	33.4 (1.0)	29.5 (1.2)	27.3 (0.77)	27.9 (1.1)
Aspect ratio	6.08 (0.17)	6.08 (0.055)	6.19 (0.11)	6.20 (0.16)
Wing mass density (mg/mm2)	0.0043 (0.001)	0.0051 (0.0002)	0.0050 (0.001)	0.0058 (0.001)
Abdomen drag (mm2)	4.93 (0.30)	3.42 (0.37)	4.91 (0.35)	3.44 (0.65)

Values are means, parentheses show standard deviations

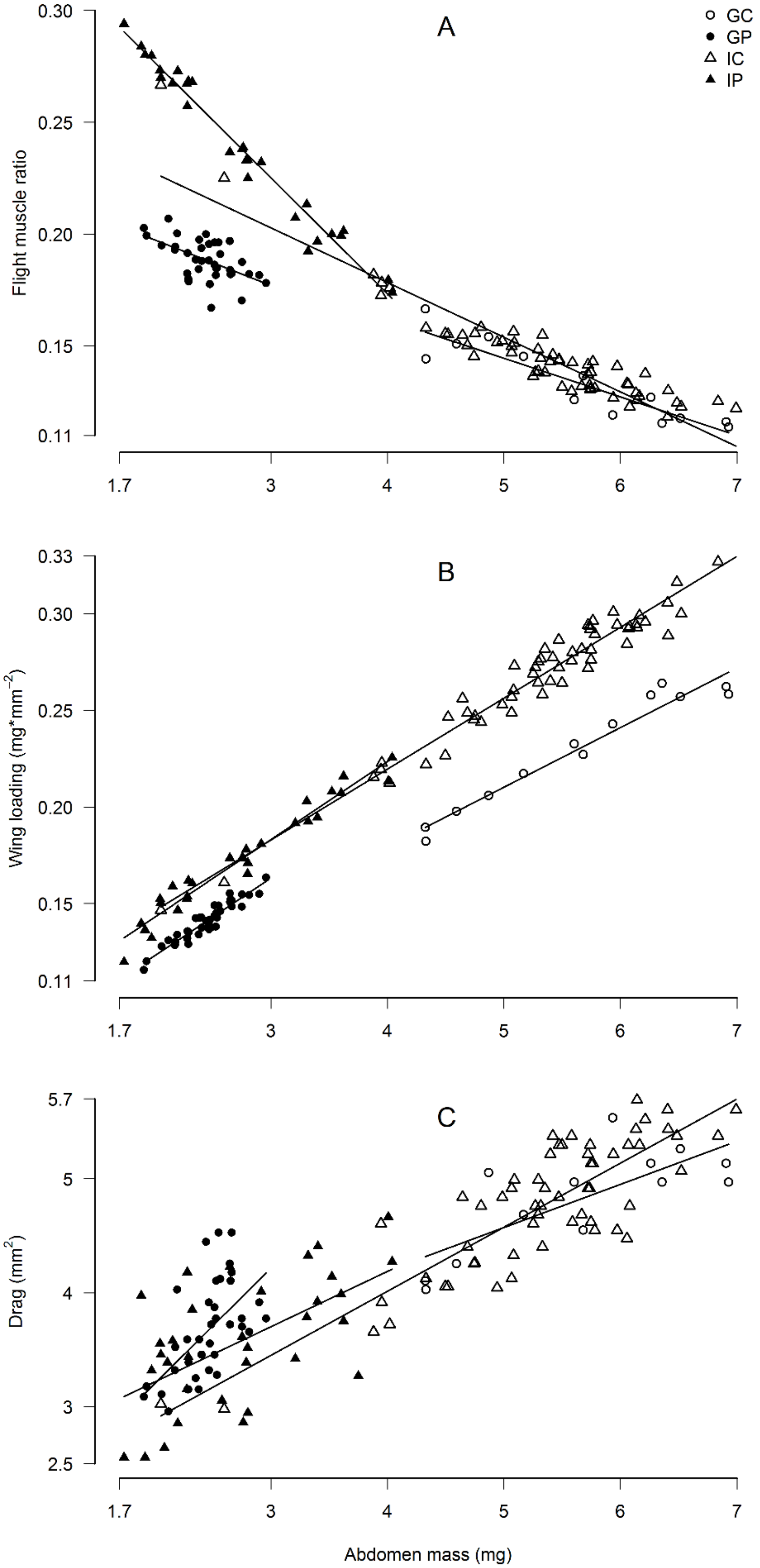
A
B
C
GC
GP
IC
IP
Flight muscle ratio
0.30
0.25
0.20
0.15
0.11
Wing loading (mg*mm^{-2})
0.33
0.30
0.25
0.20
0.15
0.11
Drag (mm^{2})
5.7
5
4
3
2.5
1.7
3
4
5
6
7
Abdomen mass (mg)

Figure 8. Queen flight morphology and abdomen mass. For all queen morphs heavier abdomens impact flight morphology by (A) decreasing flight muscle ratio, (B) increasing wing loading, and (C) increasing abdomen drag. GC = *S. geminata* claustral, GP = *S. geminata* parasitic, IC = *S. invicta* claustral, IP = *S. invicta* parasitic.

Table 7. Ordinary least squares regressions of queen flight morphology on abdomen mass

	Queen	*n*	Slope	Intercept	*r2*	*p*	Corr. *p*
FMR	GC	13	-0.0173	0.231	0.8467	8.4 x 10^{-6}	1.7 x 10^{-5}
	GP	38	-0.0205	0.238	0.3151	2.5 x 10^{-4}	2.5 x 10^{-4}
	IC	58	-0.0244	0.276	0.857	2.0 x 10^{-16}	8.0 x 10^{-16}
	IP	28	-0.0518	0.380	0.9717	2.0 x 10^{-16}	8.0 x 10^{-16}
Wing loading	GC	13	0.0309	0.0558	0.9523	1.3 x 10^{-8}	1.3 x 10^{-8}
	GP	38	0.0403	0.0423	0.8981	2.0 x 10^{-16}	8.0 x 10^{-16}
	IC	56	0.0367	0.0727	0.9482	2.0 x 10^{-16}	8.0 x 10^{-16}
	IP	28	0.0403	0.0621	0.9538	2.0 x 10^{-16}	8.0 x 10^{-16}
Drag	GC	13	0.379	2.678	0.5772	0.003	2.6 x 10^{-3}
	GP	38	1.015	1.171	0.3425	0.0001	3.4 x 10^{-4}
	IC	58	0.562	1.767	0.7258	2.0 x 10^{-16}	8.0 x 10^{-16}
	IP	33	0.485	2.247	0.3425	0.0003	6.9 x 10^{-4}

GC = claustral *S. geminata*, *GP* = parasitic *S. geminata*,
IC = claustral *S. invicta*, *IP* = parasitic *S. invicta*
Corr. p shows *p* values corrected for experimentwise error

Flight muscle ratios, wing loading and abdomen drag varied among queens types in the predicted directions. Due to their heavier abdomens flight muscle ratios (P1) of claustral *S. geminata* queens were 32% lower (0.13 ±0.018 versus 0.19 ±0.009, $p = 2.9 \times 10^{-8}$), and those of claustral *S. invicta* queens 38% lower (0.15 ±0.024 versus 0.24 ±0.035, $p = 2.0 \times 10^{-15}$), than their parasitic counterparts (Figure 9A). Likewise, wing loading (P2) was 63% higher in claustral *S. geminata* (0.230 ±0.029 versus 0.141 ±0.010 mg/mm^2, $p = 7.9 \times 10^{-8}$) and 55% higher in claustral *S. invicta* (0.268 ±0.034 versus 0.173 ±0.028 mg/mm^2, $p = 2.2 \times 10^{-16}$) than in parasitic queens of the same species (Figure 9B). Larger abdomens resulted in 32% higher drag (P3) in claustral *S. geminata* (4.82 ±0.46 versus 3.66 ±0.42 mm^2, $p = 1.7 \times 10^{-7}$) and 33% higher drag in claustral *S. invicta* (4.76 ±0.60 versus 3.58 ±0.56 mm^2, $p = 3.3 \times 10^{-14}$) than in parasitic queens (Figure 9C). The same results apply when comparing colony averages. Colony average flight muscle ratios were 32% lower in claustral *S. geminata* (0.13 ±0.005 versus 0.19 ±0.006, $p = 1.4 \times 10^{-5}$) and 42% lower in claustral *S. invicta* (0.14 ±0.0008 versus 0.24 ±0.039, $p = 0.001$) than in their parasitic counterparts (Figure 10A). Colony average wing loading was 76% higher in claustral *S. geminata* (0.239 ±0.018 versus 0.136 ±0.010 mg/mm^2, $p = 0.004$) and 63% higher in claustral *S. invicta* (0.274 ±0.013 versus 0.168 ±0.034 mg/mm^2, $p = 0.0002$) than in parasitic queens (Figure 10B). Finally, colony average drag was 44% higher in claustral *S. geminata* (4.93 ±0.30 versus 3.42 ±0.37 mm^2, $p = 0.001$) and 43% higher in claustral *S. invicta* (4.91 ±0.35 versus 3.44 ±0.65 mm^2, $p = 0.002$) than in their parasitic counterparts (Figure 10C). These flight morphology differences are robust, with large effect sizes. Nevertheless, due to the small number of colonies studied, further sampling may help refine these results.

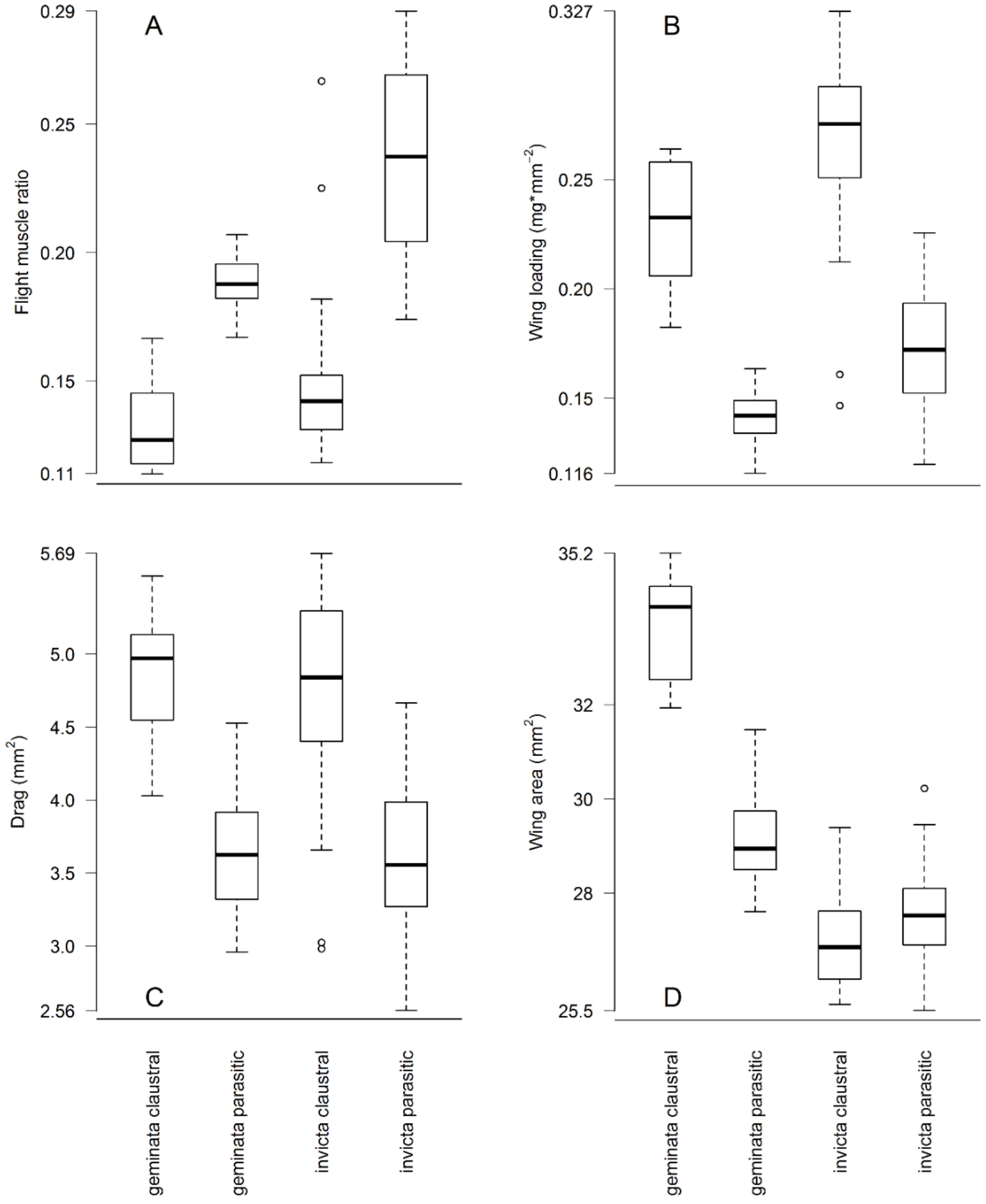

Figure 9. Claustral versus parasitic flight morphology (individual queens). Heavier abdomens mean claustral queens experience (A) lower flight muscle ratios, (B) higher wing loading, and (C) higher abdomen drag than parasitic queens of the same species. (D) Claustral founders in *S. geminata* have evolved larger wings than parasitic queens, compensating somewhat for the wing loading effects of heavier abdomens. In *S. invicta*, however, there is either no difference in wing size (see text) or claustral queens have slightly smaller wings.

Wing morphology also differed among queen types as predicted. Claustral *S. geminata* wing areas (P4) were 16% larger (33.7 ±1.1 versus 29.1 ±0.9 mm^2, $p = 1.1 \times 10^{-10}$) and their forewings 8% longer (7.19 ±0.14 versus 6.66 ±0.10 mm, $p = 6.1 \times 10^{-10}$) than in parasitic queens (Figure 9D). Similarly, when comparing colony averages, claustral *S. geminata* wings were 13% larger (33.4 ±1.0 versus 29.5 ±1.2 mm^2, $p = 0.005$) and 6% longer (7.12 ±0.14 versus 6.69 ±0.11 mm, $p = 0.016$) than in parasitic queens (Figure 10D). Claustral *S. invicta* queens (P5), in contrast, had slightly smaller (27.0 ±0.9 versus 27.6 ±1.0 mm^2, $p = 0.004$) and shorter wings (6.48 ±0.10 versus 6.57 ±0.12 mm, $p = 0.002$) than parasitic queens (Figure 9D). The differences in *S. invicta*, however, were small—only a 2% difference in wing area (0.6 mm^2) and 1% difference in forewing length (0.09 mm)—and disappeared when comparing colony averages (wing area: 27.3 ±0.77 versus 27.9 ±1.1 mm^2, $p = 0.28$, Figure 10D; forewing length: 6.49 ±0.057 versus 6.58 ±0.082 mm, $p = 0.07$). Aspect ratios did not differ among queen types in either species (*S. geminata* claustral 6.14 ±0.19 versus parasitic 6.11 ±0.10, $p = 0.57$; *S. invicta* claustral 6.23 ±0.15 versus parasitic 6.24 ±0.17, $p = 0.76$) and neither did wing mass density in *S. geminata* (claustral 0.0042 ±0.002 versus parasitic 0.0050 ±0.0006 mg/mm^2, $p = 0.09$). These similarities held when comparing colony averages. Colony average aspect ratios did not differ among queen types in either species (*S. geminata* claustral 6.08 ±0.17 versus parasitic 6.08 ±0.055, $p = 0.97$, *S. invicta* claustral 6.19 ±0.11 versus parasitic 6.20 ±0.16, $p = 0.90$), and neither did wing mass density in *S. geminata* (claustral 0.0043 ±0.001 versus parasitic 0.0051 ±0.0002 mg/mm^2, $p = 0.12$). Individual claustral *S. invicta* queens, on the other hand, had lighter wings than parasitic queens (0.0047 ±0.001 versus 0.0059 ±0.001 mg/mm^2, $p = 3.5 \times$

10^{-5}), but again the difference was slight (0.0012 mg/mm^2) and disappeared when comparing colony averages (claustral 0.0050 ±0.001 versus parasitic 0.0058 ±0.001, p = 0.06).

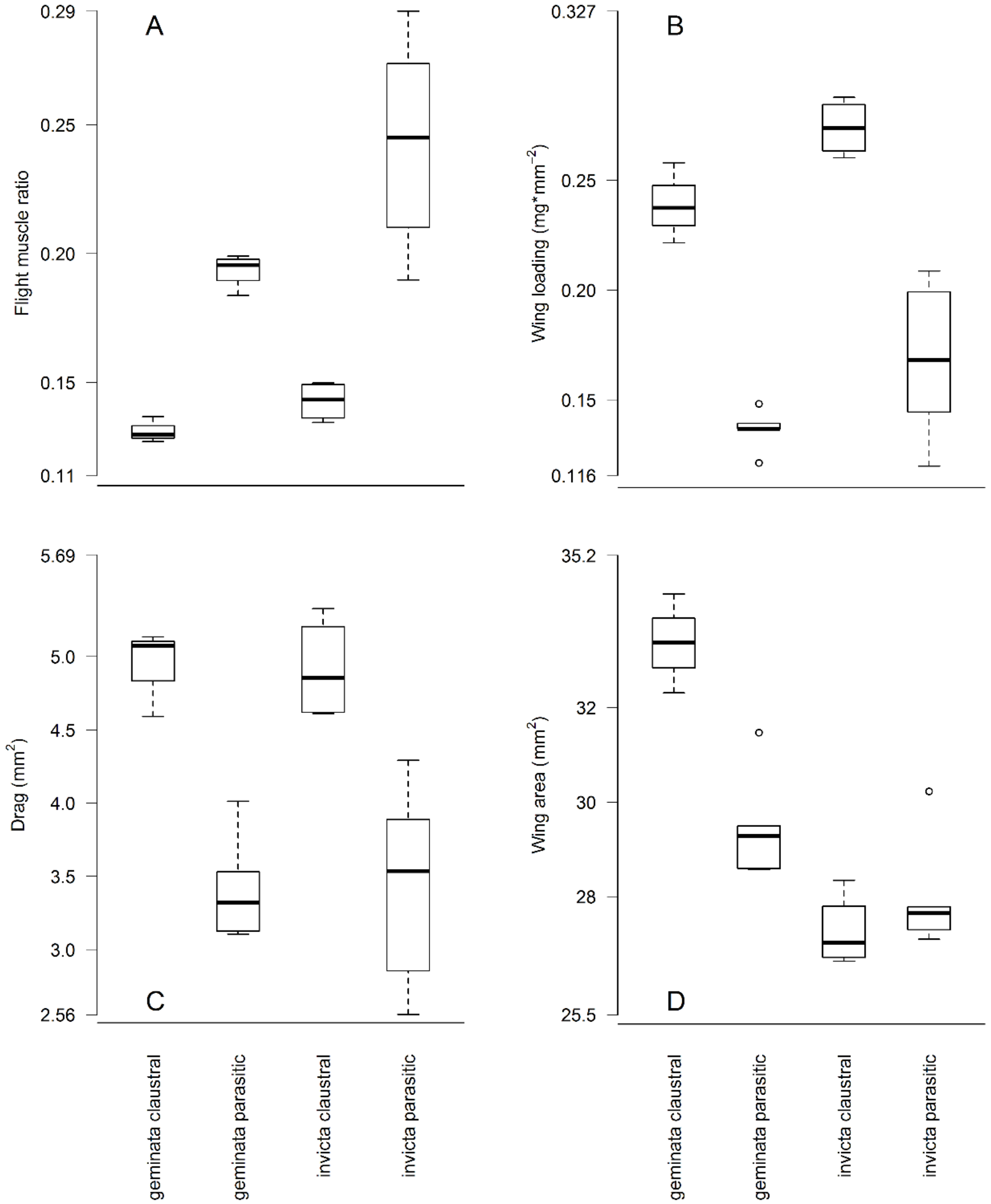

Figure 10. Claustral versus parasitic morphology (colony averages). Similar results apply when comparing colony averages. Claustral queens have (A) lower flight muscle ratios, (B) higher wing loading, and (C) higher abdomen drag than parasitic queens of the same species. (D) Claustral founders in *S. geminata* have evolved larger wings than parasitic queens, but in *S. invicta* there is no difference in wing size among queen types.

Live flight

Queens from different colonies did not differ in total flight duration (Kruskal-Wallis p = 0.45), log-transformed flight muscle ratio (ANOVA p = 0.18), abdomen mass (Kruskal-Wallis p = 0.09), or wing loading (Kruskal-Wallis p = 0.13), nor was colony identity a significant factor in any of the three quantile regressions (abdomen mass p = 0.40, flight muscle ratio p = 0.49, wing loading p = 0.76), and we therefore pooled the data. Among individual queens, the range of queen flight durations (P6) decreased with abdomen mass, so that the heaviest queens flew only for short time periods but light queens could have long or short flights (Figure 11A). When comparing maximum flight durations, the heaviest queens were able to fly only about 5% as long as the lightest (250 versus 4,900 seconds), with each milligram of abdominal loading decreasing maximum flight duration by about 18 minutes (quantile regression through upper quartile, Table 8A). Likewise, shorter maximum flight durations were associated with reduced flight muscle ratios (Figure 11B) and increased wing loading (Figure 11C), either of which, or both in combination, could be the mechanism driving shorter flight durations in heavier abdomens. Across all 32 queens, flight durations ranged over a hundredfold, from 47 seconds to over 79 minutes (ignoring one queen who flew for only four seconds). Notably, the longest flights lasted 30 to 75% longer than previous estimates of the maximum duration of *S. invicta* flights, which ranged from 45 minutes to 1 hour (Vogt et al. 2002). All measures of central tendency—least squares regressions and Spearman’s rank correlations—agreed in direction with the upper quartile results (Figure 11, Table 8B-C). As expected, however, they were not significant because minimum flight durations were the same for all flight morphologies.

Any queen can fly for short periods, and only the range and maximum vary with a queen’s ability.

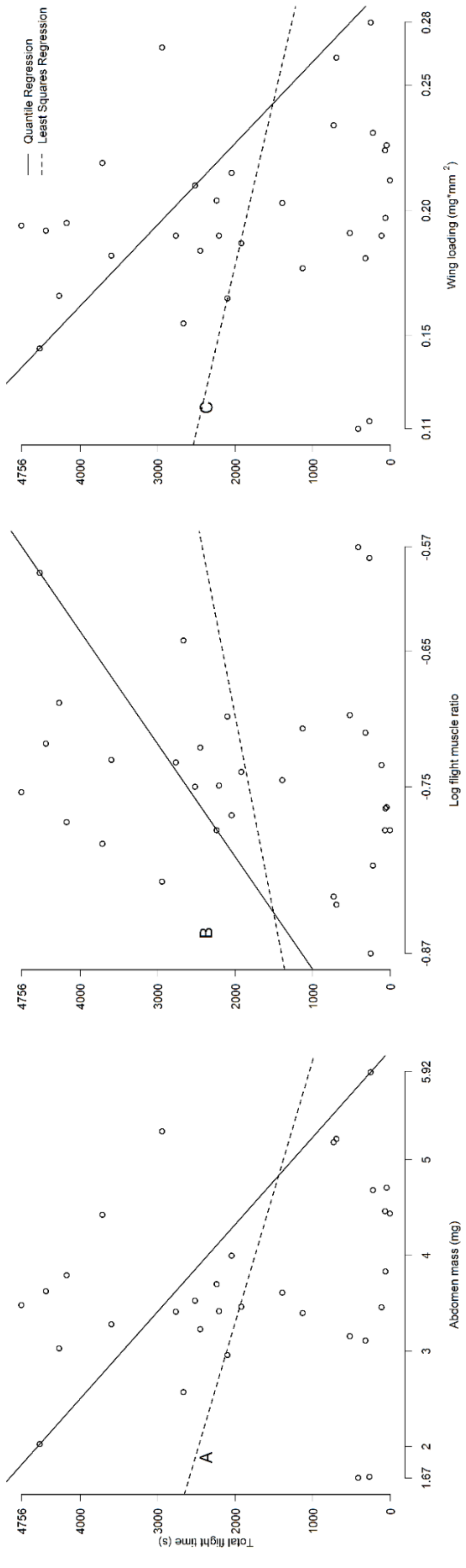
Total flight time (s)
4756
4000
3000
2000
1000
0
A
1.67
2
3
4
5
5.92
Abdomen mass (mg)
B
-0.87
-0.75
-0.65
-0.57
Log flight muscle ratio
C
0.11
0.15
0.20
0.25
0.28
Wing loading (mg*mm ^{2})
Quantile Regression
Least Squares Regression

Figure 11. Flight duration and morphology. In claustral *S. invicta* queens, (A) heavier abdomens decrease a queen's flight endurance due to (B) lower flight muscle ratios and (C) higher wing loading. Quantile regressions are through the top quartile.

Table 8. Maximum flight duration versus queen morphology

A. Quantile Regressions

	Intercept	Slope	*p*	Corr. *p*
Abdomen mass	6742.350	-1096.915	0.024	0.054
Log flight muscle ratio	11633.347	12015.789	0.031	0.054
Wing loading	8988.231	-30815.385	0.018	0.054

B. Ordinary Least Squares Regressions

	r^2	Intercept	Slope	*p*	Corr. *p*
Abdomen mass	0.05	3202.1	-364.5	0.21	0.63
Log flight muscle ratio	0.02	4377	3405	0.41	0.68
Wing loading	0.03	3345	-7552	0.34	0.68

C. Spearman's Rank Correlations

	r_s	*p*	Corr. *p*
Abdomen mass	-0.27	0.14	0.42
Log flight muscle ratio	0.22	0.22	0.42
Wing loading	-0.26	0.16	0.42

All regressions were performed on *S. invicta* (*n* = 32).
Quantile regressions are through the upper quartile.
Corr. p shows *p* values corrected for experimentwise error

Tradeoff model

Claustral and parasitic *S. invicta* queens appear to differ in their emphasis on flight versus reproduction (Figure 12). All else being equal, the heavier abdomens of claustral queens allow them to produce more workers in the early stages of colony founding, but the lighter abdomens of parasites should allow them to fly longer or farther in search of host colonies. The average claustral queen, with a 5.3 mg abdomen, would produce three times as many initial workers as the average parasitic queen with a 2.7 mg abdomen (31 versus 10 workers). At the same time, the parasitic queen should be able to fly over four times as long (3,800 versus 900 seconds) and 1.5 times as fast (0.9

versus 0.6 ms^{-1} on average), resulting in a 6-fold increase in flight range (3420 m versus 540 m). These predicted flight ranges agree with independent estimates that most claustral fire ant queens fly only a few hundred meters and for less than half an hour (Markin et al. 1971; Tschinkel 2013a).

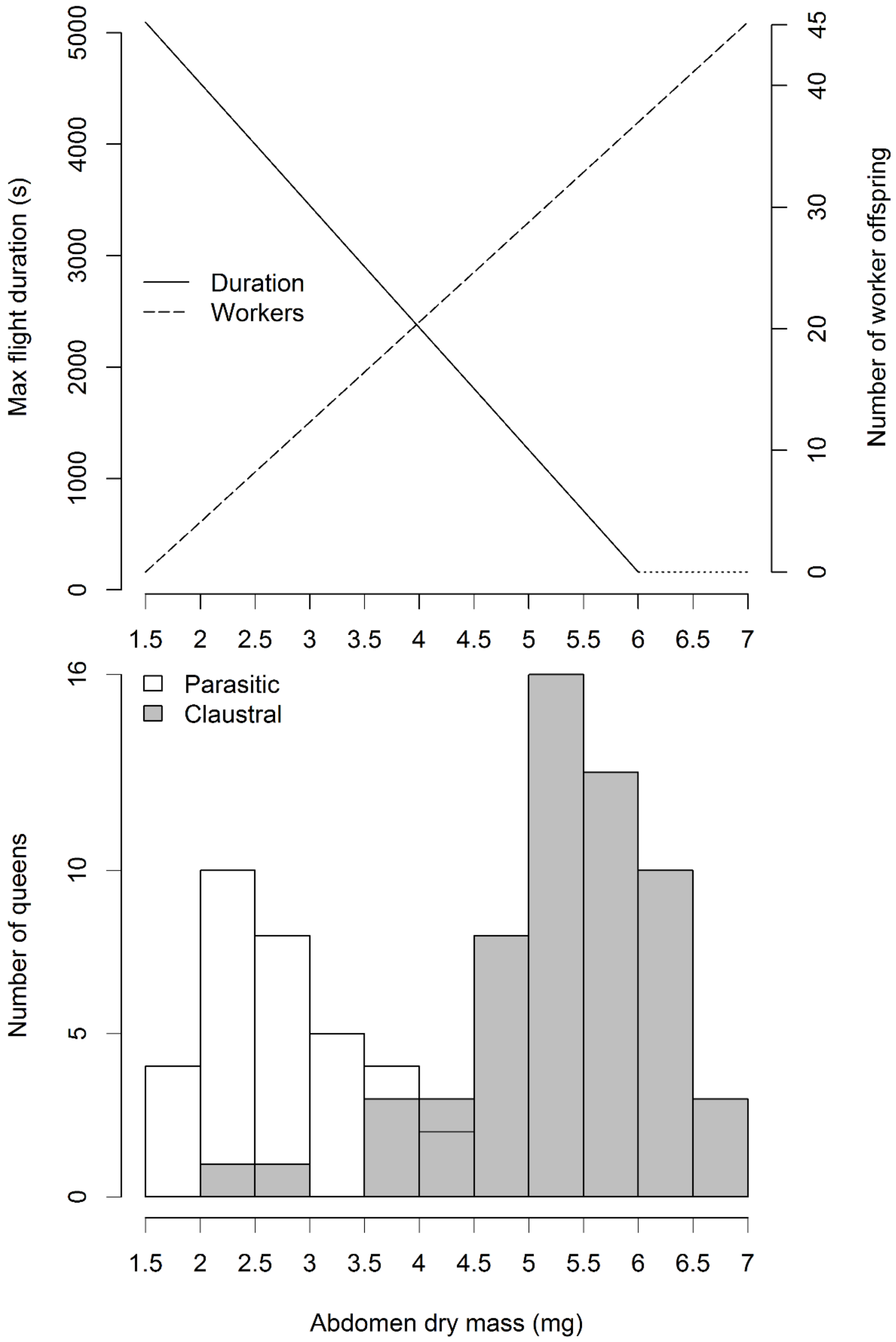
Max flight duration (s)
0
1000
2000
3000
4000
5000
Duration
Workers
Number of worker offspring
0
10
20
30
40
45
1.5
2
2.5
3
3.5
4
4.5
5
5.5
6
6.5
7
Number of queens
0
5
10
16
Parasitic
Claustral
Abdomen dry mass (mg)

Figure 12. Fire ant reproduction-dispersal tradeoff model. Ant queens experience a tradeoff between flight ability and reproductive output mediated by abdomen mass. Claustral queens sacrifice flight endurance for colony founding ability, whereas parasitic queens cannot found colonies but can fly longer in search of hosts. Flight duration curve is adapted from Figure 5A, and durations above 6 mg abdomen mass are speculative. Worker production curve is adapted from the literature (Tschinkel 1993a). Abdomen mass histograms show *S. invicta* queens from the flight morphology comparisons.

Discussion

Colonies of queen polymorphic ant species balance tradeoffs to allocate investment in different queen types. Parasitic queens are light and cheap to produce but have low reproductive outputs and cannot found colonies on their own. Claustral queens, on the other hand, are heavy and expensive but can produce many workers and found colonies independently. According to the Found or Fly hypothesis polymorphic queens should differ also in dispersal ability. Using morphological and experimental evidence, we document dispersal polymorphisms in two fire ant species, *Solenopsis geminata* and *S. invicta*. The heavier abdomens of claustral queens cause them to have 32 to 38% lower flight muscle ratios, 55 to 63% higher wing loading, and 32 to 33% higher abdomen drag than conspecific parasites. If queen castes are developmentally determined, species can respond to this tradeoff by altering the morphology of the two queens. In *S. geminata*, for example, claustral queens develop 16% larger wings than parasitic queens, offsetting some of the effects of heavier abdomens on wing loading. Heavy abdomens, through their effects on flight morphology, reduce maximum flight speed (Vogt et al. 2002) and maximum flight duration. All else being equal, claustral queens should thus have reduced flight and dispersal ability relative to their parasitic counterparts. To our knowledge, this is the first detailed study of dispersal polymorphisms among conspecific flying queen castes. Our results suggest that dispersal tradeoffs play a role in the evolution of alternative reproductive strategies and the origin of ant parasite-host systems.

Parasitic fire ant queens require specific nest sites—orphaned host colonies—that occur at low densities across the landscape (3 to 19 nests per hectare, Tschinkel 1996). Claustral queens, in contrast, can potentially found a colony in any vacant patch of soil. If parasitic queens experience greater dispersal ability, it would grant them larger search areas and more search time to locate potential hosts. Assuming a purely horizontal flight and a constant maximum flight speed of 1.5 ms^{-1} (Vogt et al. 2002), the 900 second flight of the average claustral queen would mean a potential colonization area of about 6 km^2. If the same flight relationships hold in both queen types, the average parasitic queen could fly for 3,800 seconds and have a potential colonization area of 102 km^2—a 17-fold difference. Using the lower and perhaps more realistic flight speeds of 0.9 and 0.6 ms^{-1} for parasitic and claustral queens, the difference more than doubles to 36-fold (36 versus 1 km^2). Models of the reproductive success of the two queen types (Tschinkel 1996; DeHeer & Tschinkel 1998), and the fitness return per investment for the colonies producing them, should therefore incorporate these search area differences.

Enhanced dispersal is not the only benefit associated with better flight ability. The leaner abdomens of parasitic queens should also result in greater maneuverability, which would likely increase their ability to evade predators (Chai & Srygley 1990; Fjerdingstad & Keller 2004) and to navigate aerial mating swarms to choose a mate and copulate (Davidson 1982; Fjerdingstad & Boomsma 1997). Their higher flight muscle ratios should also allow them to fly at lower temperatures than queens with heavier abdomens (Marden 2000). Indeed, in both fire ants the parasitic queens fly at cool times of the year, *S. geminata* in fall and *S. invicta* in late winter, while their claustral counterparts fly in spring and summer (McInnes & Tschinkel 1995; Tschinkel 1996).

The low weights of parasitic queens, and the apparent tradeoff between reproduction and dispersal, are probably not just an artifact of fall- and winter-reared queens being lighter due to reduced food availability. Small energy reserves are a common trait across parasitic ant species in general, regardless of when they fly (Keller & Passera 1989; Rüppell & Heinze 1999). In *S. invicta*, for which we have detailed year-round census and metabolic data, fall is actually a time of abundance and colony growth, with colony energy input exceeding expenditures (Tschinkel 1993b; Tschinkel 2013a). In fact, colonies achieve their maximum annual size and nutrient stockpile in January, just before parasitic queens leave on mating flights (Tschinkel 1993b; Tschinkel 2013a). Fire ant colonies are therefore likely able to afford large and nutrient-rich parasitic queens were it profitable to do so, especially at the low numbers in which they are produced (Morrill 1974).

Quantifying dispersal ability is rarely straightforward. For example, claustral and parasitic queens may have qualitatively different flight behaviors and experience different flight environments. In *S. invicta* claustral queens fly up into the atmosphere and may take advantage of high altitude winds (Markin et al. 1971), while parasitic queens may disperse in low searching flights along the ground where wind speeds are reduced. Strict extrapolations based on flight performance in one queen type may thus not accurately describe flight in others. Further, laboratory flight experiments may not capture natural dispersal behavior in which queens may take off from varying heights by climbing vegetation, and fly several kilometers. In interpreting our flight duration experiment we analyzed the maximum performance range of queens (the upper quartile of flight durations) and further work is needed to explain flight variation below those maximum values. Finally, other factors besides flight may limit a queen's ability to successfully disperse. In the most obvious example, parasitic queens can only disperse to areas where there are already populations of conspecifics to act as hosts, whereas claustral queens can colonize vacant habitats. More detailed field studies are needed to fully elucidate the costs and benefits of dispersal polymorphisms in these species.

Their diverse life histories and ability to generate multiple castes from the same genome make ants ideal organisms for studying morphological tradeoffs (Tschinkel 2013b; Keller et al. 2014). By positing one such tradeoff involving flight morphology, the Found or Fly hypothesis provides a framework for addressing questions of ant dispersal and the evolution of alternative reproductive strategies. In the case of parasitic species the study of flight may even grant insight into the selective forces shaping a ubiquitous pathway for sympatric speciation (West-Eberhard 2005). Although we focus on monogyne populations with only one queen per colony, similar tradeoffs may play out when comparing colonies with varying queen number (Ross & Keller 1995). Recognizing dispersal differences among all queen types, and knowing how colonies allocate investment among them, may allow us to better predict rates of range expansion in these invasive species. Investigations of male dispersal are likewise necessary for a complete understanding of gene flow and the evolution of alternative reproductive strategies in ants (Ross & Shoemaker 1997; Peeters 2012; Shik et al.

2013). In the case of females, at least, it is clear that queen types represent not only different ways to found colonies, but also different ways to fly.

Acknowledgements

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Chapter 4. Are invasive fire ants kept in check by native aerial insectivores?

This chapter has been accepted, with some modifications, to the journal *Biology Letters*.

Abstract

Aerial predator-prey interactions may impact populations of many terrestrial species. Here we use altitude loggers to study aerial foraging in a native insectivore, the Purple Martin (*Progne subis*), in the southern United States. Purple Martins fed primarily on mating queens and males of the invasive Red Imported Fire Ant (*Solenopsis invicta*), and doubled their foraging efficiency by doing so. Across the USA, Purple Martins likely eat billions of fire ant queens each year, potentially impacting the spread of this species. Alternatively, predation on fire ants may help sustain populations of Purple Martins and other aerial insectivores.

Introduction

Most terrestrial animal species fly to forage, mate, evade predators, disperse or migrate (Dudley 2000). Because most land animals fly, and all flying animals land, terrestrial and aerial food webs are linked by the movement of individuals between them. Dispersing insects, for example, occur at high densities hundreds of meters above ground and may form the bases of aerial food webs (Hardy & Milne 1938a).

Ants, as some of the most abundant animals in terrestrial environments (Hölldobler & Wilson 1990), are probably prominent players in the air as well. Although worker ants are wingless, most ant species rely on flight for reproduction (Hölldobler & Wilson 1990). Mature colonies produce winged queens and males that fly to mate and disperse, often aggregating in swarms. Ant sexuals are relatively defenseless and more nutritious than many other insects. Queens in particular, which found new colonies after mating, contain abdominal reserves of fats, proteins, and glycogen that constitute up to 70% of their body weight (Keller & Passera 1989; Hahn et al. 2004). As dense aggregations of nutritious prey, ant mating flights thus present attractive targets for predators (Whitcomb et al. 1973).

Within the United States, the invasive Red Imported Fire Ant (*Solenopsis invicta*) is a conspicuous potential prey. Native to South America, *S. invicta* was accidentally introduced to the USA in the 1930s and to other countries since, including Australia, China, and several areas in Central America and Southeast Asia (Tschinkel 2013a). In its non-native ranges *S. invicta* lives at high densities in anthropogenic or disturbed habitats. One hectare of pasture can produce ~40,000 queens per mating flight and 250,000 per year, and the resulting swarms of millions of individuals can cover thousands of square kilometers (Markin et al. 1971; Tschinkel 2013a). Fire ants have an extended breeding season in which flights occur repeatedly throughout the spring and summer, and in warm environments can happen year-round (Morrill 1974). Fire ants are perhaps one of the most abundant, nutritious and reliable food sources available to an aerial predator. Yet no native predators are known to depend on them, and release from predation probably contributes to their invasion success (Tschinkel 2013a).

We examined the value of fire ants as prey for aerial predators by tracking the foraging altitudes and prey capture of nesting Purple Martins (*Progne subis*). Purple Martins are the highest-foraging songbirds in North America. They routinely forage over 150 meters above ground (Johnston & Hardy 1962) and show up on radar up to 4,000 meters (Bridge et al. 2014). They are abundant and widespread, and capture a variety of insect prey during flight (Johnston 1967; Tarof & Brown 2013). They sometimes eat ants (Johnston 1967) and have been seen flying near *S. invicta* mating swarms (Whitcomb et al. 1973), but predation on fire ants has not been demonstrated.

Methods

We studied Purple Martins breeding in nest boxes at the University of Oklahoma Biological Station on Lake Texoma, Marshall County, Oklahoma (33° 52’ 50’’ N, 96° 48’ 02’’ W, elevation 196 m), from 27 May to 15 June 2014. This was within the mating seasons of many regional ant species (Dunn et al. 2007), including fire ants (Tschinkel 2013a).

To measure foraging altitudes, we attached altitude loggers to 25 nesting Purple Martins (13 females and 12 males). Each logger consisted of a battery, air temperature and barometric pressure sensors, a clock, memory, and a harness made of 0.7 mm ©Stretch Magic elastic cord. The entire apparatus weighed ~0.5–1.0 grams, ~1–2% of average breeding Purple Martin body weight (Tarof & Brown 2013). The loggers recorded temperature and pressure every 20 or 30 s, and we compared these readings to those made simultaneously at a weather station 22 km away at North Texas Regional Airport, Grayson County, Texas (33° 42’ 50.4’’ N, 96° 40’ 22.8’’ W, elevation 228 m). Using the weather station data as a reference, we calculated flight altitudes using the barometric formula

$$h = h_b - R^* \ln\left(\frac{P}{P_b}\right) / gM$$

where h = bird altitude (m), h_b = airport altitude (m), R^* = the universal gas constant (8.31432 N·m/(K·mol)), P = logger pressure (Pa), P_b = airport pressure (Pa), g =

standard gravitational acceleration (9.80665 m/s^2), and M = the molar mass of air (0.0289644 kg/mol).

When examining profiles of altitude over time, peaks represent aerial foraging trips and troughs show when parents returned to nest level, often to deliver prey to nestlings (Figure 13). Altitude readings at nest level fluctuated slowly about ±15 m throughout the day, likely due to slightly different weather conditions between our study site and the weather station. To correct for fluctuations, we subtracted adjacent trough altitudes from foraging altitudes, giving accurate measurements of foraging height relative to nest level. Trough altitudes were set at 5 m (the height of the nest boxes) and foraging heights were adjusted accordingly.

While we logged flight altitudes we also retrieved prey delivered to nestlings by logged parents. To prevent nestlings from swallowing the prey, we fitted them with neck collars (Poulsen & Aebischer 1995) made from 0.7 mm ©Stretch Magic elastic cord. We then monitored the nest for several hours per day between 0630 and 1830. When a parent delivered a bolus of prey we retrieved it from the chicks' mouths and preserved it in 95% ethanol. We then loosened the collars, fed the chicks mealworms (*Tenebrio molitor*), and re-fastened their collars. Prey items were counted and identified to species or morphospecies. To estimate biomass we dried 1–20 specimens of each species for 48 hours at 60–65 °C and weighed them to 0.001 mg. We multiplied each species' average dry mass by the number of individuals to estimate total biomass. Species captured only once were excluded from biomass analyses.

To avoid collaring fledglings or injuring young chicks, we targeted nests with chicks ~1–3 weeks old, out of a 28 day nestling period (Tarof & Brown 2013). We began nest monitoring and prey retrieval a day after attaching the loggers. Overall we monitored 13 nests for 1–4 days each, 12 of which yielded prey samples.

To determine prey altitudes, we associated each prey with a foraging trip by matching the delivery time to a trough in the altitude profile (Figure 13). The peak between this point and the most recent prior trough described the foraging trip in which the prey was captured. We then determined the trip’s duration and maximum height above ground. We excluded from duration analyses three trips with unclear durations.

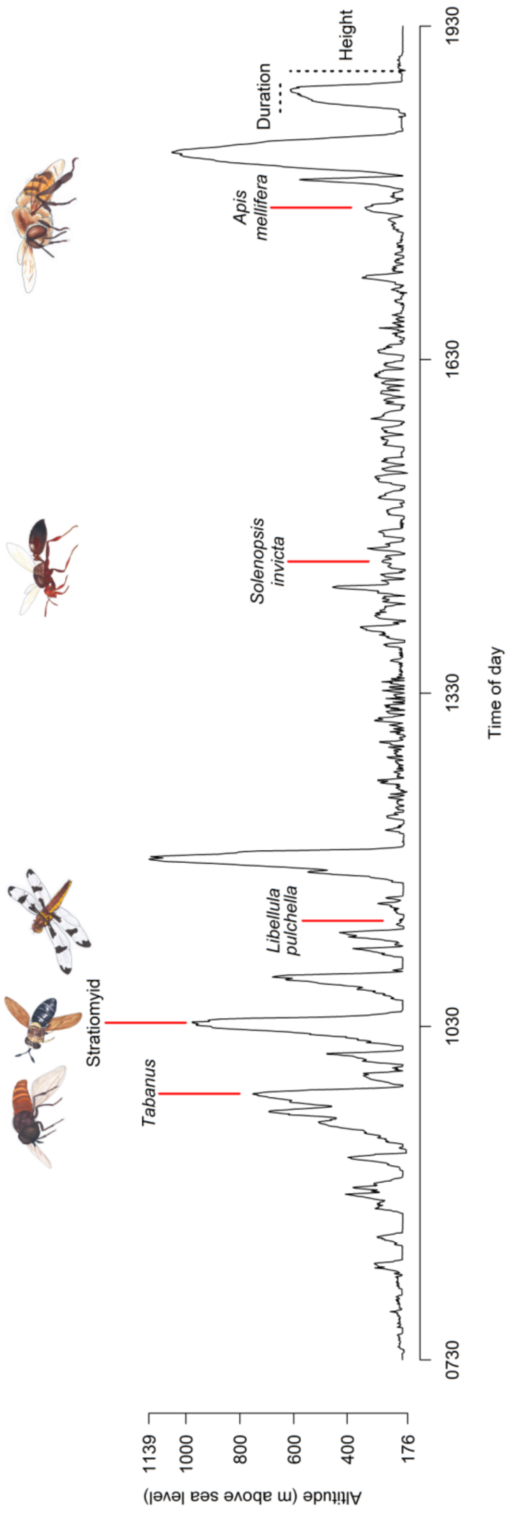

Figure 13. Example Purple Martin altitude profile. We calculated maximum heights and durations of foraging trips that were matched to prey delivered to nestlings.

Analyses were performed in the program R (R Core Team 2012). We logged ~911 hrs of altitude data (2.2–87.7 per bird, average 43.4 ±20.4). We collected prey from 311 foraging trips, 86 of which were matched to altitude data. Six birds did not provision nestlings and nine loggers failed during the observation periods, leaving ten birds with combined altitude and prey data. We compared data collection and flight behavior of male and female Purple Martins using *t*-tests for normal and Kruskal-Wallis tests for non-normal data. We checked normality with Shapiro-Wilk tests. The number of altitude-matched foraging trips did not vary with sex (K-W *P* = 0.91, *n* = 4 females and 6 males, female median 3.5 trips, interquartile range 2.5–9.5, male 6.5 trips, interquartile range 1.5–12.0), nor did maximum flight heights (*t*-test *P* = 0.41, *n* = 10 females and 11 males, female mean 780 ±198 m, male 912 ±466) or foraging heights (*t*-test *P* = 0.58, *n* = 4 females and 6 males, female mean 78 ±56 m, male 105 ±93). We therefore pooled the data.

We examined the efficiency of fire ants as prey using an optimal foraging approach, in which more efficient prey yield higher reward per search effort. We predicted that the higher nutrient content and abundance of fire ants compared to other insects should result in shorter Purple Martin foraging times. We tested this using ANCOVAs of foraging trip duration by prey type, with maximum trip height as a covariate. We limited analyses to foraging trips below the maximum height at which a fire ant of either sex was captured (≤163 m, *n* = 71). We checked for changes in foraging efficiency throughout the day by including prey delivery time as a covariate. We detected no time effect, however (*P* = 0.2084), and excluded it from final analyses.

Results and discussion

In 311 foraging trips the Purple Martins captured 3,765 individuals of 79 species, including 8 ants (Table 9). *Solenopsis invicta* was the dominant prey species no matter how they were ranked. Fire ants were captured on 32% of foraging trips, and made up 56% of prey items and 27% of biomass fed to nestlings. The queens alone constituted 29% of foraging trips, 31% of prey items and 22% of biomass. Fire ants were delivered to 9 of 12 nests from which we collected prey, to chicks of all ages. The three nests that did not receive fire ants were monitored during five days of dry weather in which no mating flights occurred. Half of all birds with altitude-matched foraging trips captured fire ants, and both sexes did so at similar frequencies (males: 3/6 birds, 12/52 foraging trips (23.1%); females: 2/4 birds, 7/34 trips (20.1%)). No other species approached this prominence in the Purple Martin diet.

Table 9. Prey items delivered to Purple Martin nestlings

Species	**Individuals**	**(%)**	**Trips**	**(%)**	**Avg mass (mg)**	**Total mass (mg)**	**(%)**	**Max height (m)**	**Median height (m)**
Total	**3765**		**311**		**37.097**	**37908.750**		**162.1**	**95.7**
Ants	**2973**	**78.96**	**131**	**42.1**	**10.075**	**14242.813**	**37.57**	**79.8**	**42.5**
Camponotus pennsylvanicus F	33	0.88	20	6.4	62.786	2071.941	5.47	39.3	14.9
Camponotus pennsylvanicus M	13	0.35	3	1.0	7.534	97.945	0.26	*NA*	*NA*
Crematogaster laeviuscula F	62	1.65	14	4.5	7.505	465.281	1.23	75.5	48.8
Crematogaster laeviuscula M	4	0.11	2	0.6	0.506	2.023	0.01	NA	NA
Dorymyrmex flavus F	610	16.20	12	3.9	2.080	1268.931	3.35	105.2	46.0
Dorymyrmex flavus M	144	3.82	10	3.2	0.081	11.609	0.03	111.0	49.6
Formica pallidefulva F	1	0.03	1	0.3	19.514	19.514	0.05	*NA*	*NA*
Lasius neoniger F	2	0.05	2	0.6	1.222	2.443	0.01	22.3	8.6
Monomorium minimum F	1	0.03	1	0.3	*NA*	*NA*	*NA*	*NA*	*NA*
Solenopsis invicta F	1152	30.60	89	28.6	7.338	8452.928	22.30	78.3	42.1
Solenopsis invicta M	950	25.23	77	24.8	1.947	1849.888	4.88	88.1	47.1
Temnothorax sp. F	1	0.03	1	0.3	0.311	0.311	0.00	118.5	83.2
Termites									
Reticulitermes sp.	**308**	**8.18**	**3**	**1.0**	**0.763**	**235.136**	**0.62**	**22.3**	**8.6**

Flies	**172**	**4.57**	**74**	**23.8**	**18.264**	**3898.470**	**10.28**	**196.2**	**100.6**
Asilid sp. 1	2	0.05	2	0.6	7.878	15.756	0.04	*NA*	*NA*
Asilid sp. 2	4	0.11	4	1.3	66.452	265.807	0.70	176.5	38.0
Calliphorid sp.	2	0.05	2	0.6	9.370	18.740	0.05	*NA*	*NA*
Chironomid sp.	2	0.05	2	0.6	0.363	0.726	0.00	89.2	66.8
Culicid sp.	32	0.85	3	1.0	0.360	11.509	0.03	116.4	60.6
Stratiomyid sp. 1	3	0.08	3	1.0	30.714	92.141	0.24	787.8	273.7
Stratiomyid sp. 2	2	0.05	2	0.6	8.960	17.920	0.05	*NA*	*NA*
Stratiomyid sp. 3	1	0.03	1	0.3	*NA*	*NA*	*NA*	28.8	11.3
Syrphid sp.	3	0.08	3	1.0	20.168	60.504	0.16	507.7	416.5
Tabanus sp.	68	1.81	30	9.6	35.551	2417.451	6.38	120.7	55.2
Tachinid sp. 1	27	0.72	20	6.4	22.864	617.315	1.63	81.1	50.2
Tachinid sp. 2	10	0.27	9	2.9	33.096	330.963	0.87	17.6	12.9
Tachinid sp. 3	2	0.05	2	0.6	18.050	36.100	0.10	*NA*	*NA*
Tachinid sp. 4	5	0.13	1	0.3	0.468	2.340	0.01	*NA*	*NA*
Tephritid sp.	1	0.03	1	0.3	*NA*	*NA*	*NA*	116.4	60.6
Tipulid sp.	8	0.21	2	0.6	1.400	11.197	0.03	116.4	60.6
Hemipterans	**126**	**3.35**	**68**	**21.9**	**22.435**	**4467.104**	**11.78**	**256.1**	**180.5**
Alydid sp.	3	0.08	3	1.0	14.259	42.776	0.11	922.4	761.4
Belostomatid sp.	2	0.05	2	0.6	72.041	144.082	0.38	59.5	20.4
Cercopid sp.	1	0.03	1	0.3	*NA*	*NA*	*NA*	*NA*	*NA*
Cicadellid sp. 1	18	0.48	10	3.2	2.653	47.758	0.13	23.6	10.5
Cicadellid sp. 2	4	0.11	3	1.0	1.365	5.460	0.01	922.4	761.4
Cicadellid sp. 3	1	0.03	1	0.3	*NA*	*NA*	*NA*	*NA*	*NA*
Cicadellid sp. 4	1	0.03	1	0.3	*NA*	*NA*	*NA*	*NA*	*NA*
Cicadellid sp. 5	2	0.05	1	0.3	3.364	6.728	0.02	116.4	60.6
Cicadellid sp. 6	1	0.03	1	0.3	*NA*	*NA*	*NA*	22.3	8.6
Coreid sp.	1	0.03	1	0.3	*NA*	*NA*	*NA*	93.1	71.6
Corixid sp.	5	0.13	2	0.6	2.107	10.535	0.03	116.4	60.6
Gerrid sp.	5	0.13	2	0.6	5.376	26.878	0.07	128.2	78.8
Leptoglossus sp.	48	1.27	36	11.6	73.672	3536.232	9.33	91.2	59.5
Mirid sp. 1	5	0.13	4	1.3	3.384	16.921	0.04	*NA*	*NA*
Mirid sp. 2	1	0.03	1	0.3	*NA*	*NA*	*NA*	*NA*	*NA*
Notonectid sp.	1	0.03	1	0.3	*NA*	*NA*	*NA*	*NA*	*NA*
Pentatomid sp. 1	7	0.19	6	1.9	38.692	270.842	0.71	72.0	13.9
Pentatomid sp. 2	2	0.05	2	0.6	28.603	57.206	0.15	116.4	60.6
Pentatomid sp. 3	3	0.08	3	1.0	43.292	129.876	0.34	*NA*	*NA*
Pentatomid sp. 4	1	0.03	1	0.3	*NA*	*NA*	*NA*	922.4	761.4
Psyllid sp.	1	0.03	1	0.3	*NA*	*NA*	*NA*	*NA*	*NA*
Reduviid sp.	5	0.13	2	0.6	2.606	13.028	0.03	116.4	60.6
Scutellarid sp. 1	7	0.19	6	1.9	22.683	158.783	0.42	137.6	45.3
Scutellarid sp. 2	1	0.03	1	0.3	*NA*	*NA*	*NA*	236.6	53.3
Dragonflies & damselflies	**58**	**1.54**	**54**	**17.4**	**119.428**	**6574.541**	**17.34**	**75.4**	**36.1**
Anax junius	7	0.19	7	2.3	324.101	2268.704	5.98	17.9	16.0
Enallagma sp.	9	0.24	8	2.6	12.386	111.476	0.29	57.2	33.4
Erythrodiplax umbrata	1	0.03	1	0.3	*NA*	*NA*	*NA*	28.8	11.3
Gomphid spp.	14	0.37	14	4.5	152.398	2133.567	5.63	54.3	24.2
Libellula luctuosa	1	0.03	1	0.3	*NA*	*NA*	*NA*	*NA*	*NA*
Libellula pulchella	1	0.03	1	0.3	*NA*	*NA*	*NA*	31.5	17.3
Pachydiplax longipennis	7	0.19	7	2.3	75.784	530.486	1.40	*NA*	*NA*
Perithemis tenera	5	0.13	5	1.6	24.282	121.410	0.32	68.6	18.9
Sympetrum corruptum	7	0.19	7	2.3	86.843	607.898	1.60	272.1	112.2
Tramea lacerata	5	0.13	5	1.6	160.200	801.002	2.11	73.0	55.8
Unidentified dragonfly remains	1	0.03	1	0.3	*NA*	*NA*	*NA*	*NA*	*NA*

Bees & wasps	**55**	**1.46**	**28**	**9.0**	**63.110**	**2995.178**	**7.90**	**105.4**	**47.7**
Apis mellifera M	49	1.30	25	8.0	53.923	2642.235	6.97	105.4	47.7
Apis mellifera Worker	2	0.05	2	0.6	53.281	106.562	0.28	*NA*	*NA*
Apid sp. queen	1	0.03	1	0.3	*NA*	*NA*	*NA*	*NA*	*NA*
Sphecid sp.	3	0.08	3	1.0	82.127	246.381	0.65	*NA*	*NA*
Grasshoppers									
Melanoplus sp.	**35**	**0.93**	**32**	**10.3**	**110.780**	**3877.283**	**10.23**	**142.4**	**58.3**
Moths	**26**	**0.69**	**25**	**8.0**	**84.429**	**1599.673**	**4.22**	**207.1**	**107.0**
Noctuid sp. 1	13	0.35	12	3.9	49.067	637.870	1.68	86.8	45.2
Noctuid sp. 2	1	0.03	1	0.3	*NA*	*NA*	*NA*	*NA*	*NA*
Noctuid sp. 3	7	0.19	7	2.3	110.673	774.710	2.04	375.3	152.1
Noctuid sp. 4	2	0.05	2	0.6	93.547	187.094	0.49	450.4	276.8
Noctuid sp. 5	1	0.03	1	0.3	*NA*	*NA*	*NA*	90.9	39.3
Noctuid sp. 6	1	0.03	1	0.3	*NA*	*NA*	*NA*	128.2	78.8
Noctuid sp. 7	1	0.03	1	0.3	*NA*	*NA*	*NA*	111.0	49.6
Beetles	**7**	**0.19**	**7**	**2.3**	**5.230**	**15.690**	**0.04**	**32.1**	**16.9**
Carabid sp.	1	0.03	1	0.3	*NA*	*NA*	*NA*	*NA*	*NA*
Curculionid sp.	1	0.03	1	0.3	*NA*	*NA*	*NA*	23.6	10.5
Scarabaeid sp. 1	1	0.03	1	0.3	*NA*	*NA*	*NA*	*NA*	*NA*
Scarabaeid sp. 2	3	0.08	3	1.0	5.230	15.690	0.04	40.5	23.2
Unidentified beetle	1	0.03	1	0.3	NA	NA	NA	*NA*	*NA*
Lacewings	**3**	**0.08**	**1**	**0.3**	**1.431**	**2.862**	**0.01**	**116.4**	**60.6**
Chrysopid sp.	1	0.03	1	0.3	*NA*	*NA*	*NA*	116.4	60.6
Hemerobiid sp.	2	0.05	1	0.3	1.431	2.862	0.01	116.4	60.6
Cockroaches									
Blatellid sp.	**1**	**0.03**	**1**	**0.3**	***NA***	***NA***	***NA***	***NA***	***NA***
Caddisflies									
Unidentified caddisfly	**1**	**0.03**	**1**	**0.3**	***NA***	***NA***	***NA***	***NA***	***NA***

% shows percent of total. Trip percentages add up to more than 100% because more than one prey type can be caught per trip. Masses are dry weights. Biomass percentages ignore singleton species where no voucher was weighed.
Bolded summary rows show sums and averages for the following insect group
Some prey data, but not flight altitudes, are included in an unpublished manuscript submitted elsewhere

Purple Martins also doubled their foraging efficiency by targeting fire ant queens. Foraging trips in which fire ant queens were captured lasted 1–8 min shorter than those targeting other prey types at similar altitudes, resulting in a ~50% decrease in foraging time (Figure 14, Table 10). Considering trips in which fire ants of either sex were captured yielded similar but more variable results (Table 10), suggesting that foraging efficiency increases are driven by nutrient-packed queens rather than males. Efficiency increases are apparent even after excluding exceptionally long trips (>2,000 seconds, queens *P* = 0.002, either sex *P* = 0.001). Among 27 short trips (<500 seconds) which did not target fire ants, 31 species were captured, none more than six times.

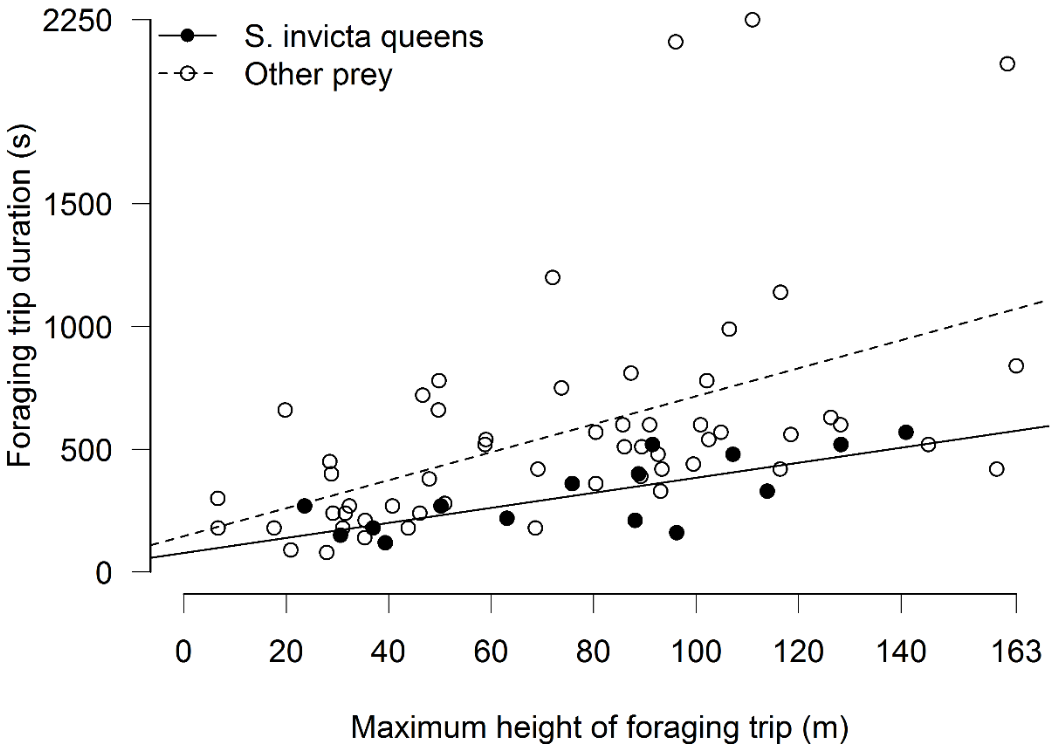

Figure 14. Foraging durations and flight altitudes. Each point represents a foraging trip which resulted in delivery of a bolus of prey. Filled circles show foraging trips in which fire ant queens were captured, open circles show those in which only other insects were captured.

Table 10. Purple Martin foraging trip durations by prey type and flight altitude. A) ANCOVA results comparing durations of trips in which fire ants were captured to those targeting only other insects; B) OLS regressions of duration versus altitude.

A. *ANCOVA*	Factor	df	F	*P*
Trip duration (queens)	Max height	1	22.093	<0.001
	Prey type	1	6.880	0.011
	Error	68		
Trip duration (queens & males)	Max height	1	22.782	<0.001
	Prey type	1	9.214	0.003
	Error	68		

B. *Regressions* Duration vs. max height	*n*	Intercept	Slope	r^2
S. invicta queens	15	77.57	3.06	0.55
Other prey	56	146.49	5.69	0.25
S. invicta queens & males	19	139.75	2.50	0.30
Other prey	52	110.09	6.47	0.40

Purple Martins and fire ants overlap in range across ~1.5 million km^2 of the USA (Figure 15) (Sauer et al. 2014; USDA 2015). We observed Purple Martins delivering 1,152 *S. invicta* queens to nestlings over 38 logger-days, an average of 30.3 queens per day per parent. If Purple Martins behave similarly elsewhere, then conservatively assuming one million nesting pairs in the introduced range of *S. invicta* (Kelly et al. 2013), over a 28 day nestling period their chicks would consume 1.7 billion fire ant queens—each queen a potential new colony. Adding predation by adults, the number likely amounts to at least tens of billions per year.

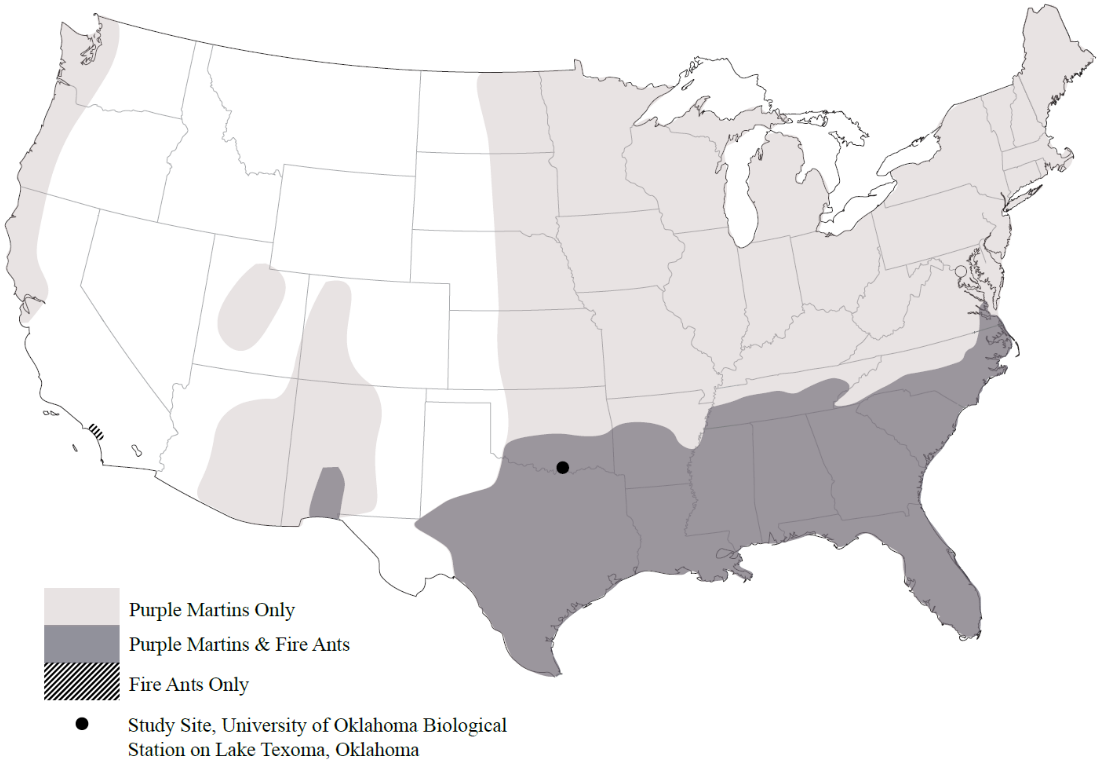

Figure 15. Purple Martin and Red Imported Fire Ant ranges. The two species overlap across the Southeastern United States. (Range maps adapted from Sauer et al. 2014 and USDA 2015).

It is unknown whether this intense predation limits fire ant population densities or range expansion. It is likewise unclear if consumption of fire ant queens affects predator physiology, as their bodies contain several compounds used in chemical communication and defense (Tschinkel 2013a). Nevertheless, by doubling foraging efficiency, predation on fire ants may boost Purple Martin populations. Purple Martins in most of the USA rely on man-made nest boxes for which they compete with other species (Tarof & Brown 2013). Over the past 50 years Purple Martin populations have declined across the northern USA and Canada for unknown reasons, but are stable or increasing in southern regions colonized by *S. invicta* (Michel et al. 2015). We suggest that southern populations are likely subsidized by the introduced prey.

Fire ants are probably a valuable prey for other native insectivores as well. Populations of Barn Swallows (*Hirundo rustica*) and Northern Rough-winged Swallows (*Stelgidopteryx serripennis*) are also stable or increasing within the introduced range of *S. invicta* despite declines elsewhere (Michel et al. 2015). Chimney Swifts (*Chaetura pelagica*), Tree Swallows (*Tachycineta bicolor*), and Eastern Kingbirds (*Tyrannus tyrannus*) may feed opportunistically on *S. invicta* queens (Whitcomb et al. 1973). We observed four additional species foraging in *S. invicta* swarms—Scissor-tailed Flycatchers (*Tyrannus forficatus*), Western Kingbirds (*Tyrannus verticalis*), Great-tailed Grackles (*Quiscalus mexicanus*), and Red-headed Woodpeckers (*Melanerpes erythrocephalus*).

Solenopsis invicta is often viewed as an invasive species with negative impacts on animal communities (Wojcik et al. 2001), but to some aerial predators its arrival represents a windfall of abundant, reliable and nutritious food. Like Purple Martins in the USA, aerial predators in other regions where *S. invicta* has been introduced may likewise take advantage of this newfound prey. Purple Martins possess substantial ecological and economic value because of the insects they consume and the nest-box industry that maintains them (Kelly et al. 2013). The value of fire ants as prey for these and other avian insectivores should be accounted for when planning costly control efforts (Tschinkel 2013a).

Ethics

This research followed all applicable laws and was approved by the University of Oklahoma Institutional Animal Care and Use Committee (protocol R12-019C, Appendix).

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Chapter 5. Predator foraging altitudes reveal the structure of aerial insect communities

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This chapter has been submitted, with some modifications, to the journal *Scientific Reports*

Abstract

The atmosphere is populated by a diverse array of dispersing insects and their predators. We study aerial insect communities by tracking the foraging altitudes of an avian insectivore, the Purple Martin (*Progne subis*). By attaching altitude loggers to nesting Purple Martins and collecting prey delivered to nestlings, we determined the flight altitudes of ants and other insects. We then test hypotheses relating ant body size and reproductive ecology to flight altitude. Purple Martins flew up to 1,889 meters above ground, and nestling provisioning trips ranged up to 922 meters. Insect communities were structured by body size such that species of all sizes flew near the ground but only light insects flew to the highest altitudes. Ant maximum flight altitudes decreased by 60% from the lightest to the heaviest species. Winged sexuals of social insects (ants, honey bees, and termites) dominated the Purple Martin diet, making up 88% of prey individuals and 45% of prey biomass. By transferring energy from terrestrial to aerial food webs, mating swarms of ants and other social insects likely play a substantial role in atmospheric ecosystems. Although we focus on Purple Martins and ants, our method could be applied to a range of aerial communities.

Introduction

Most terrestrial animal species (Wagner & Liebherr 1992; Dudley 2000), many plants (Gurevitch et al. 2006), and countless microorganisms (Womack et al. 2010) enter the Earth's skies to forage, mate, evade predators, disperse or migrate. They use the atmosphere as habitat (Chilson et al. 2012; Diehl 2013). Insects in particular occur at high densities in the atmosphere, where they form the base of aerial food webs and take advantage of high altitude winds to disperse long distances (Hardy & Milne 1938a, b; Freeman 1945; Johnson 1957; Srygley & Dudley 2008). Because most land animals fly, and all flying animals land, most terrestrial communities may be impacted by events that play out in the sky. Atmospheric ecosystems remain little understood, however, largely because of their inaccessibility to humans. Tracking devices and radar may reveal the movements of large birds, bats and insects as they travel through the atmosphere but most flying animals are too small for such methods (Bridge et al. 2011;

Chapman et al. 2011; Kays et al. 2015). We remain mostly ignorant about the composition and physical structure of aerial insect communities.

Ants, for example, are one of the most abundant and influential animal groups in terrestrial environments (Hölldobler & Wilson 1990; Folgarait 1998; Agosti et al. 2000), but their role in the atmosphere is almost entirely unknown. Most of the world's 12,000+ ant species (Bolton et al. 2006; AntWeb 2013) enter the atmosphere to reproduce and disperse (Vogt et al. 2000; Peeters & Ito 2001). Mature colonies produce winged queens and males that fly from the nest to find mates and new nest sites, often aggregating in high altitude mating swarms or leks that in some species may contain millions of individuals (Hölldobler & Wilson 1990; Markin et al. 1971; Shik et al. 2013). Ant sexuals are relatively defenseless, and the bodies of queens contain large reserves (up to 70% body weight) of fats, storage proteins, and glycogen that help them found new colonies (Keller & Passera 1989; Hahn et al. 2004). Ant sexuals are therefore an abundant and nutritious target for predators (Moser 1967; Whitcomb et al. 1973; Hespenheide 1975), and their mating swarms may provide substantial nutrient inputs into aerial food webs.

The abundance and diversity of ants, their value as prey for aerial predators, and their occasional spread across non-native ranges as exotic species (Holway et al. 2002) highlight the importance of understanding how they disperse through the atmosphere. Aerial ant communities are probably structured primarily by body size, as lighter insects tend to fly at higher altitudes than heavy ones (Hardy & Milne 1938a, b; Freeman 1945; Hespenheide 1977). Lighter insects generally have lower wing loading, allowing them to stay aloft longer, fly in lower density air, and be more easily transported in rising air currents (Dudley 2000; Dillon et al. 2006; Srygley & Dudley 2008). In ants this relationship is captured by the Found or Fly Hypothesis, which posits a tradeoff between flight ability and abdominal nutrient storage (Helms & Kaspari 2014). This view predicts that lighter ant species should be able to fly higher and take advantage of high altitude winds for dispersal, although at a presumed cost in competitive or reproductive ability once they land (Helms & Kaspari 2015). In addition to biomechanical constraints, flight altitudes may also be influenced by mating strategy. Some ant species practice a female-calling strategy in which queens mate on the ground near their home nest, rather than in aerial mating swarms, and are thought to fly only short distances (Peeters & Ito 2001; Hölldobler & Bartz 1985). According to this mating strategy view, female-calling species should fly close to the ground regardless of body weight. Altitudinal predictions of these hypotheses remain untested, however, as the small size of ants has precluded comparative studies of their flight altitudes. Here we bypass weight limitations and study aerial ant communities indirectly by following their avian predators, which are larger and more amenable to tracking, as they forage in the atmosphere.

The Purple Martin (*Progne subis*), North America's largest swallow, is an ideal predator for sampling aerial insect communities. They are abundant and widespread in the United States and Canada, and eat a wide variety of insect prey, including ants, which they capture during flight (Beal 1918; Johnston 1967; Tarof & Brown 2013). They routinely forage over 150 meters above ground (Johnston & Hardy 1962) and have been detected with weather radar up to 4,000 meters (Bridge et al. 2014), making them North America's highest-foraging songbird. We are, however, ignorant of the altitudes at which they catch different prey species. Indeed, we lack these data for any flying species, as individual altitude logging devices have only recently been developed (Spivey & Bishop 2014; Bishop et al. 2015).

Here we use ultra-lightweight altitude loggers to track the flight altitudes of nesting Purple Martins as they forage in the atmosphere. By simultaneously monitoring the prey delivered to nestlings, we also determine the identities, abundance, and flight altitudes of ants and other insects captured by the birds. We then examine aerial ant communities in the context of hypotheses relating body weight and mating strategy to flight altitudes. We believe this is the first study to log the atmospheric foraging altitudes of any bird, and although we focus on Purple Martins and ants, our method would be easily transferrable to other aerial predators and prey.

Methods

Location and dates

We studied Purple Martins breeding in nest boxes at the University of Oklahoma Biological Station on Lake Texoma, Marshall County, Oklahoma (33° 52’ 50’’ N, 96° 48’ 02’’ W, elevation 196 meters), from 27 May to 15 June 2014. These dates fall within the mating seasons of Purple Martins (Tarof & Brown 2013) and of many temperate North American ant species (Dunn et al. 2007).

Foraging altitudes

To measure the foraging altitudes of Purple Martins, we attached altitude loggers to 25 nesting adults (13 females and 12 males). Each logger consisted of a battery, air temperature and barometric pressure sensors, a clock, and memory. We trapped birds at their nest and mounted the loggers on them using a harness made of 0.7 mm ©Stretch

Magic elastic cord and crimp beads. A combined logger and harness weighed ~0.5-1.0 grams, about 1-2% of the average breeding Purple Martin body weight (Tarof & Brown 2013), and would be light enough to use on ~90% of bird species (Bridge et al. 2011). The loggers recorded air temperature and pressure every 20 or 30 s. After 1 to 4 days of recording we retrieved the loggers and downloaded the data. We then compared the pressure readings to those made simultaneously at a weather station 22 km away at the North Texas Regional Airport, Grayson County, Texas (33° 42’ 50.4’’ N, 96° 40’ 22.8’’ W, elevation 228 m). Using the weather station data as a reference, we converted the logger data to altitude measures using the barometric formula

$$h = h_b - R^{*}\ln(\frac{P}{P_b})/gM$$

where h = Purple Martin altitude (m), h_b = airport altitude (m), R^{*} = the universal gas constant (8.31432 N·m/(K·mol)), P = logger pressure (Pa), P_b = airport pressure (Pa), g = standard gravitational acceleration (9.80665 m/s^2), and M = the molar mass of air (0.0289644 kg/mol).

We thus obtained for each Purple Martin a continuous profile of altitude by time throughout the logging period (Figure 16). Peaks in the altitude profiles represent foraging trips in which a parent flew into the atmosphere to hunt prey. Likewise, troughs between the peaks indicate times when the parents returned to nest level, often to deliver prey to nestlings. The altitude readings at nest level fluctuated slowly about ±15 m throughout the day, likely due to slightly different weather conditions between our study site and the weather station used as a baseline. To correct for these fluctuations, altitudes within peaks were adjusted by subtracting the altitude of the nearest trough, giving us accurate measurements of foraging height relative to nest level. Trough heights were then assigned a value of 5 m above ground (the approximate height of the nest boxes) and the foraging heights adjusted likewise.

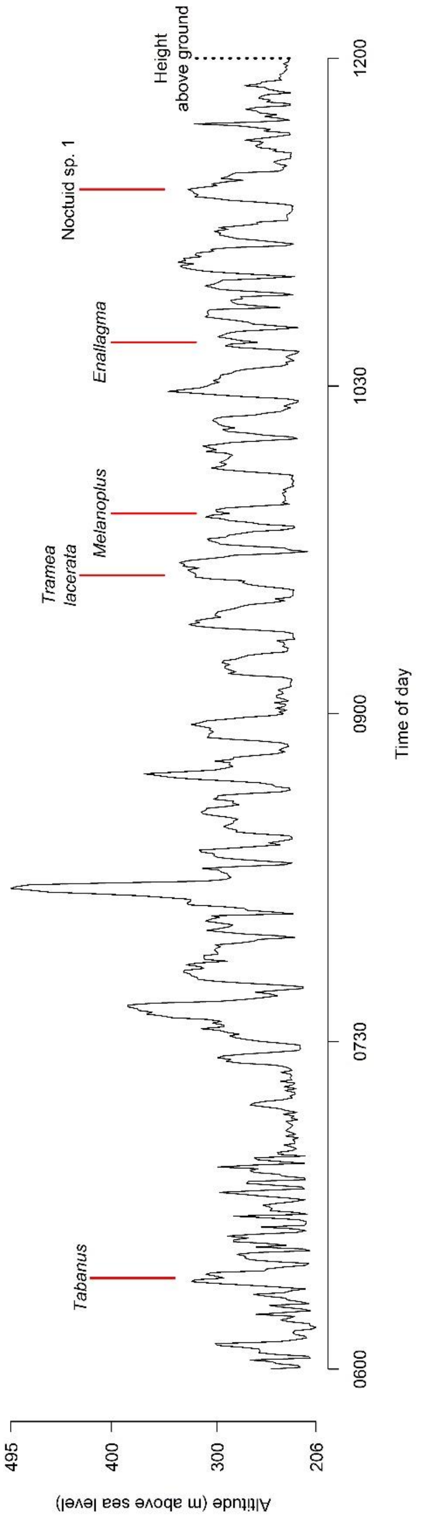

Figure 16. Example Purple Martin altitude profile. Red lines show examples of prey captured during foraging trips and delivered to nestlings. We calculated heights above ground by subtracting foraging altitudes from those at nest level.

Prey collection

While the loggers collected foraging altitude data, we retrieved all the prey that logged parents delivered to their nestlings. To prevent the nestlings from swallowing the prey, we fitted them with neck collars. Each neck collar was an adjustable noose made from 0.7 mm ©Stretch Magic elastic cord and crimp beads. After placing neck collars on the chicks we monitored the nest for several hours each day ranging from 0630 to 1830. Whenever a logged parent returned from a foraging trip to deliver prey we recorded the time and waited for the parent to leave the nest on the next foraging trip. We then entered the nest, located the chicks that had been fed, retrieved the insect prey from their mouths, and preserved the prey in 95% ethanol. We then loosened the nestlings' collars, fed them mealworms (*Tenebrio molitor*) in place of their original prey, re-fastened their neck collars and returned them to the nest. Collected prey items were later sorted and counted. Voucher specimens of each prey type were pinned and identified. We treated male and female ants separately because they are sexually dimorphic, the females weighing from 4 to 26 times as much as males (Table 9). To obtain prey biomass estimates we set aside, when possible, 1 to 20 specimens of each prey species as weight vouchers. Weight voucher specimens were dried for 48 hours at 60-65 °C and weighed to the nearest 0.001 mg. We did not weigh vouchers from 29 species captured only once each, and these were excluded from prey biomass analyses.

Nest selection and animal care

This research followed all applicable laws and was approved by the University of Oklahoma Institutional Animal Care and Use Committee (protocol R12-019C, Appendix). Purple Martins are particularly tolerant of human disturbance (Tarof & Brown 2013), and the use of neck collars in diet studies is an established method that does not usually harm nestlings (Poulsen & Aebischer 1995). Nevertheless, to avoid injuring young chicks and to prevent older chicks from fledging with the neck collars still on, we targeted nests with chicks about 1 to 3 weeks old, in the middle of their 28 day development period (Allen & Nice 1952). We avoided nests stressed by heavy mite infestations and tried to target nests with active parents that regularly delivered prey. To give parents time to acclimate to disturbance, we waited until the day after we attached the loggers to begin nest monitoring and prey retrieval. Each nest was monitored only once, for a period ranging from 1 to 4 days. During the night and whenever we were not actively monitoring nests, we loosened the neck collars to allow the chicks to feed normally on prey delivered by parents. Overall we monitored 13 nests from 5 nest boxes containing 65 chicks (average 5 per nest, range 3 to 7).

Prey altitudes

To determine the flight altitudes of prey species, we associated each prey delivery event with a foraging trip in the altitude profile. Each prey delivery time was matched to a trough in the altitude profile of the corresponding parent—the point where the foraging bird returned from the atmosphere to nest level (Figure 16). Working backward from this point, we located the most recent previous point at which the parent was at nest level. The altitude peak between these two points described the foraging trip in which the prey was captured. For each foraging trip we determined the duration, maximum height above ground, and median height above ground. We used median foraging heights, as opposed to means, because the altitudes within a foraging trip were not normally distributed. In three cases it was unclear when the foraging trip began because of continuous flight near ground level. To obtain summary statistics for prey types (Table 9), we averaged maximum height and median height across all foraging trips in which each species was captured.

Prey abundance

To further investigate how aerial ant communities are structured, we determined how their abundance varied with altitude. The number of ants of a given species delivered to nestlings from a single foraging trip ranged from 1 to 106 individuals. To the extent that catch size is determined by prey availability, this variation should reflect ant abundance at a particular foraging altitude. For all captured ant species for which we had altitude data (6 species, 8 treating males and females separately, Table 9), we pooled individuals into 10-m altitude bins based on the median foraging altitude of the trip in which they were captured. We then compared the altitudinal distributions of all species to create a preliminary map of the aerial ant community.

Data analysis

All analyses were performed in R (R Core Team 2012). Variables were tested for normality using the Shapiro-Wilk test. Paired comparisons used *t*-tests for normal and Kruskal-Wallis tests for non-normal data. Normal data are reported as means with standard deviations, non-normal data as medians with interquartile ranges.

Results

Foraging altitudes

We logged 25 birds, 13 females and 12 males, for a total of 3,279,510 s or 911 hrs. Four loggers failed completely and did not record any flight altitudes. Among the 21 logging events that were at least partly successful, the logging time per bird ranged from 7,980 s (2.2 hrs) to 315,690 s (87.7 hrs), with an average of 156,167 (±73,397) s or 43.4 (±20.4) hrs. We collected prey from 311 nestling provisioning trips, 86 of which were matched to altitude data. Fifteen of the 25 birds yielded no altitudinal foraging data, 9 because of partial or complete logger failure and 6 because they did not provision their nestlings during the observation period. The 10 birds with both altitude and foraging data provided a median of 3.5 altitude-logged foraging trips per bird (interquartile range 1.5 to 12.0), with a maximum of 26 trips.

We detected no sex differences in data collection or flight behavior and pooled the data. Males and females did not differ in the number of altitude-logged foraging trips (K-W $P = 0.91$, $n = 4$ females and 6 males, female median 3.5 trips, interquartile range 2.5 to 9.5, male 6.5 trips, interquartile range 1.5 to 12.0), maximum flight heights (t-test $P = 0.41$, $n = 10$ females and 11 males, female mean 780 ±198 m, male 912 ±466 m), maximum foraging heights (t-test $P = 0.58$, $n = 4$ females and 6 males, female mean 78 ±56 m, male 105 ±93 m), or median foraging heights (t-test $P = 0.76$, $n = 4$ females and 6 males, female mean 43±27 m, male 50 ±45 m).

Purple Martins flew to an average maximum height per bird of 849 ±362 m, with an overall maximum of 1,889 m in one male. The maximum heights of nestling provisioning trips ranged from 7 to 922 m above ground (median 88, interquartile range 41 to 116, Figure 17), and median heights from 6 to 761 m (median 38, interquartile range 22 to 67). Foraging trips lasted from 80 to 2,250 s, with a median duration of 480 s (interquartile range 270 to 735, excluding 3 trips with unclear durations $n = 83$). As would be expected, foraging trips to higher altitudes lasted longer (duration (s) = 410.8 + 1.3462 * max height (m), $n = 83$, $r^2 = 0.32$).

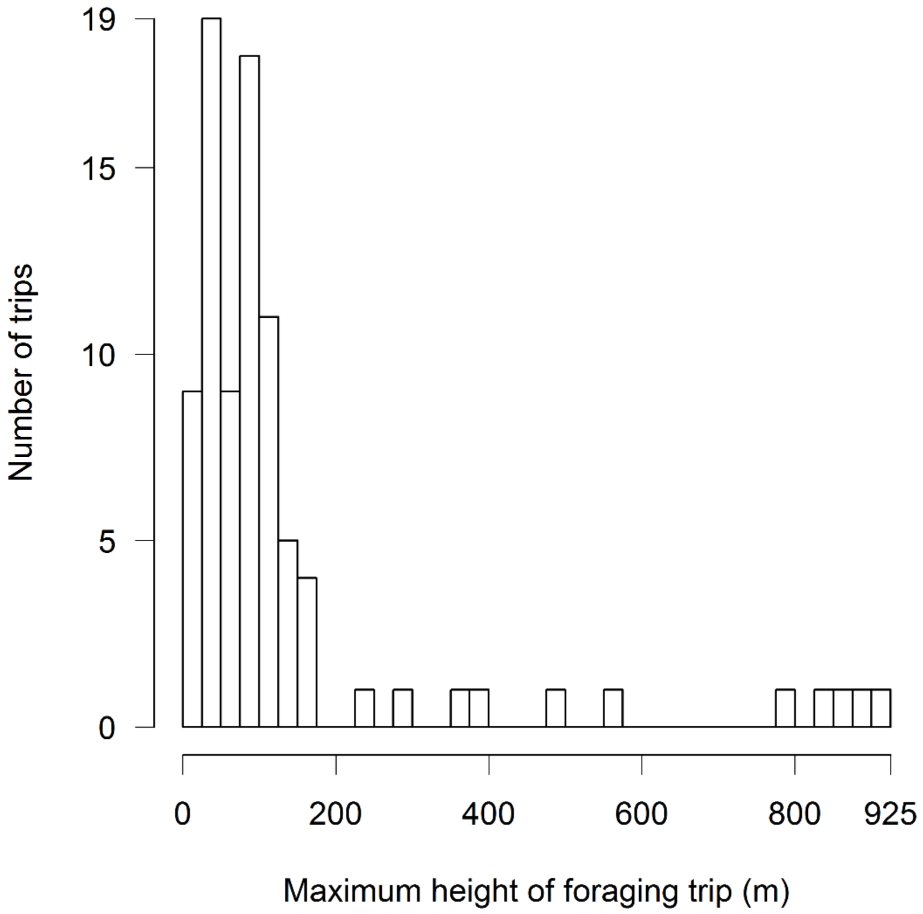

Figure 17. Purple Martin foraging altitudes

Prey altitudes

The 86 altitude-logged foraging trips yielded 56 prey species (including 8 ants, counting males and females separately) and 153 prey by altitude records, captured at up to 922 m above ground. We weighed vouchers of 43 of those 56 prey types. As predicted, flight altitudes of prey species were constrained by body mass, such that low altitudes were populated by species of any weight, but only light species were captured at the highest altitudes (Figure 18A). Ants were captured throughout the lower atmosphere up to a maximum foraging height of ~160 m. There was no relationship across all ants between average maximum flight altitudes and body weight. One outlier, however, the cornfield ant *Lasius neoniger*, is likely a female-calling species and does not engage in high altitude mating flights. Queens of two closely related species, *L. alienus* and *L. niger*, fly short distances only a few meters above ground and land to choose mates, copulate, and walk to new nest sites (Imai 1966; Bartels 1985). *Lasius neoniger* probably exhibits similar behavior and was unique in our study in being both light and low-flying, found only below ~22 m. Excluding that outlier, maximum flight altitudes decreased with body weight as predicted by the Found or Fly hypothesis. Over a 63 mg range in dry weight, maximum flight altitudes decreased by 60% ($r^2 = 0.88$, $P < 0.002$, Figure 18B). The lightest ants—male pyramid ants (*Dorymyrmex flavus*) and *Temnothorax sp.* queens—flew over 100 m above ground whereas heavy carpenter ant queens (*Camponotus pennsylvanicus*) flew under 40 m. A similar relationship held for median flight altitudes (ln median flight altitude = -0.021 * body weight + 4.01, $r^2 = 0.86$, $P < 0.003$, again excluding *L. neoniger*). The pattern was not just driven by the outlying weight of the heaviest species (*C. pennsylvanicus*), as we got similar results after log-transforming body weights so they were normally distributed (log maximum flight altitude = -0.15 * ln body weight + 4.55, $r^2 = 0.78$, $P < 0.008$, log median flight altitude = -0.18 * ln body weight + 3.91, $r^2 = 0.58$, $P < 0.05$).

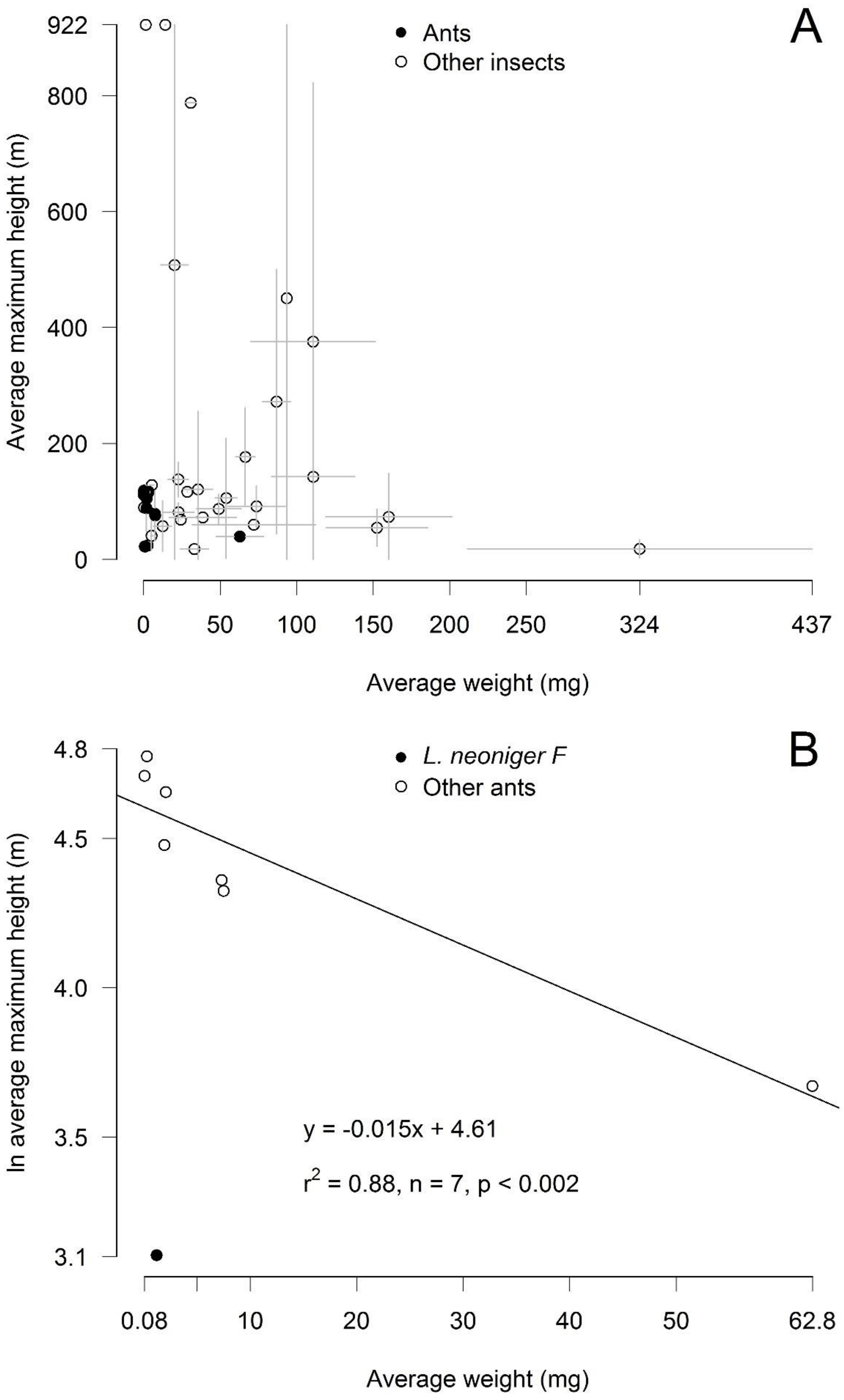
A
Ants
Other insects
Average maximum height (m)
922
800
600
400
200
0
Average weight (mg)
0
50
100
150
200
250
324
437
B
L. neoniger F
Other ants
ln average maximum height (m)
4.8
4.5
4.0
3.5
3.1
$y = -0.015x + 4.61$
$r^2 = 0.88, n = 7, p < 0.002$
Average weight (mg)
0.08
10
20
30
40
50
62.8

Figure 18. Insect flight altitudes by body size. Each point represents a single species, except ants in which dimorphic males and females are treated separately. Weights are average dry weights of voucher specimens, altitudes are averages of the maximum heights of foraging trips in which a species was captured, and error bars show standard deviations where available. *A*) Insect flight altitudes were constrained by body mass such that species of all sizes flew near the ground, but only light species flew high in the atmosphere. *B*) Among ants, lighter species flew higher in the atmosphere. The one exception—the female cornfield ant *Lasius neoniger*—is a female-calling species that does not engage in aerial mating flights.

Prey choice

We collected 83 prey species (including 12 ants, counting males and females separately, Table 9), but the diet was dominated by just a few common prey items. The Purple Martins appeared to target social insect mating swarms and aerial leks. Sexuals of social insects—ants, termites and male honey bees—were captured on over 46% of foraging trips and made up over 88% of prey individuals and over 45% of prey biomass. Ants were by far the most important of these groups, making up 42% of foraging trips, 79% of prey items, and 38% of total biomass.

Prey abundance

Ours is the first study to compare the flight altitudes of multiple ant species. Among the 86 foraging trips for which we successfully logged altitudes, several species (*Camponotus pennsylvanicus, Dorymyrmex flavus*, *Lasius neoniger,* and *Temnothorax sp.*) were collected rarely or within a single altitude (Figure 19). Two species, however, were collected across a range of altitudes. Queens of the acrobat ant *Crematogaster laeviuscula* peaked at a median foraging height of 20 m, dropping off to sporadic occurrences up to 90 m. In *Solenopsis invicta,* on the other hand, both sexes showed more or less continuous high abundances across the lower 80 m of the atmosphere. *Solenopsis invicta*, the Red Imported Fire Ant, is an exotic species that lives at high densities in its introduced range (Tschinkel 2013a). Their abundance in the air likely reflects their dominance on land. We cannot rule out, however, the possibility that this apparent high abundance is an artifact of Purple Martin dietary preferences.

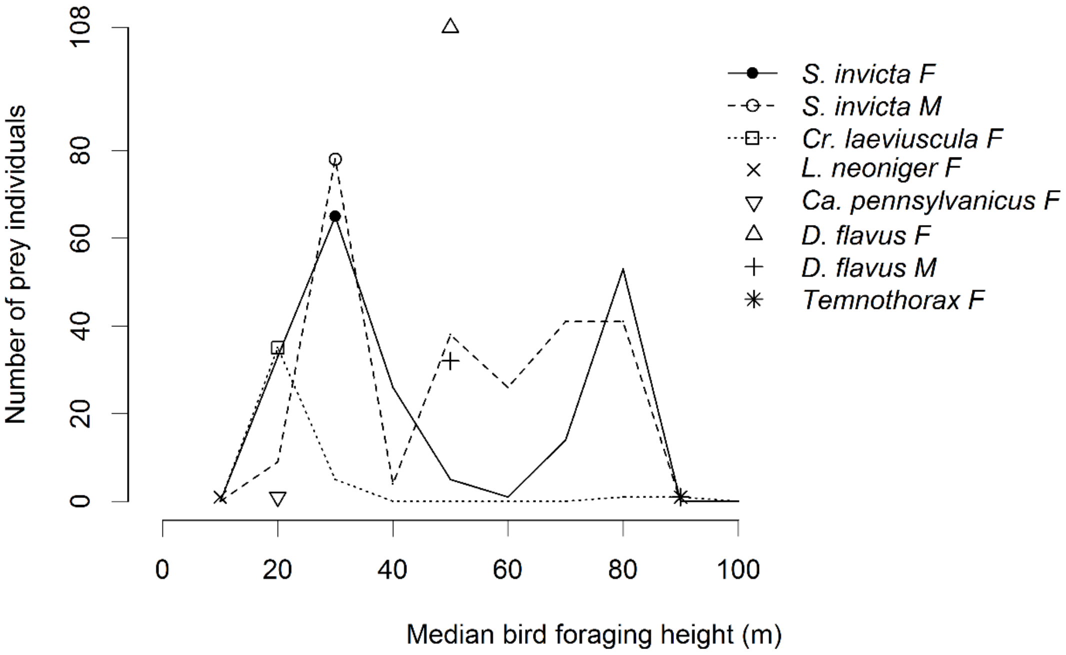

Figure 19. Ant abundance (as the number of individuals captured) varied with Purple Martin foraging altitude. Symbols show the peak abundance of each species. Two species that were captured over a range of altitudes—*Solenopsis invicta* and *Crematogaster laeviuscula*—are also represented by lines.

Discussion

The atmosphere is habitat for diverse communities of mating or dispersing insects. Purple Martins and other predators ascend hundreds of meters above the ground to take advantage of these high altitude prey resources. In viewing vertebrate predators as sampling tools, we gain a practical method for studying both the composition of aerial insect communities and the roles of insects in aerial food webs. Insect species are distributed in the air according to body size such that lighter species can occur at higher altitudes. Among aerially mating ants, for example, body size accounted for nearly 90% of variation in flight altitude. Mirroring their dominance on land, winged sexuals of ants and other social insects also play a prominent role in aerial food webs as abundant and nutritious prey.

The role of flight in ant biology is often overlooked because most individual ants are wingless workers that live in earthbound colonies. Our results, in contrast, emphasize a role for ants and other social insects as major players in atmospheric ecosystems. The lower atmosphere is populated by a diverse and temporally variable ant community structured by body size, mating strategy, and potentially other ecological drivers. Dozens or even hundreds of ant species may fly over a single location each month (Dunn et al. 2007; Kaspari et al. 2001b; Torres et al. 2001). Queens of different species vary by four orders of magnitude in body mass (Helms & Kaspari 2015), fly at different times throughout the day and night (Torres et al. 2001), and occur at altitudes ranging from ground level to hundreds of meters into the atmosphere, providing a diverse menu for aerial predators. Ant mating flights represent a large and steady flow of readily available energy and biomass from colonies on the ground to predators in the sky, and thereby link terrestrial and atmospheric food webs.

Terrestrial-atmospheric linkages like these are a common feature of life on Earth because most terrestrial animals fly or otherwise occupy the atmosphere, but none spend their entire lives in the air. We thus expect many terrestrial populations to be affected by unseen predation events in the atmosphere. By combining predator foraging altitudes with prey delivery data, we gain a unique insight into these high altitude species interactions. Although we focus on Purple Martins and ants, our method could be applied to most other flying vertebrates and their prey to gain a broader understanding of aerial ecology. Doing so is especially urgent in light of rapid human alterations to the atmosphere through air transportation, the construction of wind turbines and communication towers, and changes in weather patterns and climate.

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Chapter 6. Range expansion and reproductive polymorphisms in invasive fire ants

Abstract

Many species are expanding their ranges in response to climate changes or species introductions. Expansion-related selection likely drives the evolution of dispersal and reproductive traits, especially in invasive species introduced into novel habitats. We used an agent-based model to investigate these relationships in the red imported fire ant, *Solenopsis invicta*, by tracking simulated populations over 25 years. Most colonies of this invasive species produce two types of queens practicing alternate reproductive strategies. Claustral queens found new colonies in vacant habitats, while parasitic queens take over existing colonies whose queens have died. We investigated how relative investment in the two queen types affects population demography, habitat occupancy, and range expansion. We found that parasitic queens extend the ecological lifespan of colonies, thereby increasing a population's average colony size by up to 92%, territory size by up to 133%, and habitat occupancy by up to 12%. At the same time, investment in parasitic queens slowed the rate of range expansion by diverting investment from claustral queens. Divergent selection regimes caused edge and interior populations to evolve different levels of reproductive investment, from 40–50% investment in parasitic queens in the interior to only 4% at the edge. Our results highlight factors shaping ant life histories, including the evolution of social parasitism, and have implications for the response of species to range shifts.

Introduction

Many species throughout the world are shifting or expanding their ranges in response to climate changes or species introductions (Parmesan et al. 1999; Hickling et al. 2006; Chen et al. 2011). Range shifts may in turn drive evolutionary changes, as populations colonize vacant habitats or experience novel conditions (Thomas et al. 2001; Sexton et al. 2009). Populations at expanding range edges, in particular, are likely to evolve greater dispersal ability as a result of both selection and assortative mating (Cwynar & MacDonald 1987; Phillips et al. 2008; Hill et al. 2011). Other traits that are linked to dispersal, such as fecundity or mating system, may also evolve in response to range expansion (Burton et al. 2010; Hargreaves & Eckert 2014).

Ants present some of the world's most conspicuous recent range expansions. Many species are global invasives whose non-native ranges are expanding through natural and human-assisted dispersal (Holway et al. 2002). Colonies of most ant species reproduce and disperse by rearing winged queens that fly to locate mates and new nest sites (Hölldobler & Wilson 1990, Peeters & Ito 2001). There are countless variants of this life cycle (Heinze & Tsuji 1995; Heinze 2008), and many ants pursue multiple reproductive strategies (Ross & Keller 1995; Sundström 1995; Heinze & Keller 2000). In some species, for example, colonies can produce two different types of queens from the same genome—an independent one that founds new colonies and a parasitic one that joins existing colonies of the same species (Bourke & Franks 1991; Rüppell & Heinze 1999). These alternate strategies result in dispersal differences, since only one queen type can colonize vacant sites while the other can reproduce only in occupied areas. Reproductive polymorphisms have been documented in many invasive ants (Yamauchi & Ogata 1995; Holway et al. 2002; Tsutsui & Suarez 2003), and trait variability has been linked to invasion success in several other taxa (Richards et al. 2006; Davidson et al. 2011; Forsman 2014; González-Suárez et al. 2015). But it remains unclear how reproduction-dispersal polymorphisms in ants affect rates of range expansion, or how investment in different strategies responds to expansion-related selection.

The red imported fire ant (*Solenopsis invicta*), perhaps the best-known invasive ant, is an ideal organism for examining these relationships. It is native to South America but was accidentally introduced to the southeastern USA in the 1930s and to several other countries afterward (Tschinkel 2013). It has been expanding its non-native ranges ever since through human transport and natural dispersal during mating flights (Tschinkel 2013). Most populations of *S. invicta* are monogyne, with a single reproductive queen per colony (Porter et al. 1997). Mature monogyne colonies reproduce using both claustral and parasitic queens (Tschinkel 1996; DeHeer & Tschinkel 1998). Claustral queens fly in spring and summer and found new colonies independently. They dig nest cavities in unoccupied soil, lay eggs, and rear a first generation of workers from their own energy reserves. The parasitic queens, in contrast, fly in late winter and take over conspecific colonies whose queens have recently died, thereby inheriting an existing workforce. Parasitic queens make up a minority of a colony's reproductive effort (Morrill 1974), but are thought to provide a substantial return per investment due to the constant natural orphaning of colonies in mature populations (DeHeer & Tschinkel 1998).

Using *S. invicta* as a model, this study addresses two questions related to range expansion and alternative reproductive strategies. The first question, posed from the perspective of a population ecologist, asks how investment in parasitic queens affects the spatial distributions of fire ant populations with regard to colony size, territory size, and the propensity to expand into suitable habitats. The second question takes an evolutionary perspective and asks what the optimal relative investment in the two strategies is for colonies seeking to maximize their contribution to future generations.

The presence of parasitic queens in a population makes colonies potentially immortal. Genetic lineages within a colony are replaced over time as queens die and new ones take over. But the colony itself may remain on the landscape for generations, as long as it is successfully parasitized every time it is orphaned. This scenario prompted us to conceive the *Immortality Hypothesis*, which entails three predictions associated with extending the ecological lifespan of colonies. First, parasitic queens should increase the average colony size in a landscape. Second, parasitic queens should increase occupancy of the habitat by fire ant colonies (Korzukhin & Porter 1994). Third, in expanding ranges, investment in parasitic queens should slow range expansion by diverting investment from claustral queens that can colonize vacant sites. Alternatively, investment in parasitic queens may speed up range expansion by increasing the average size and persistence of colonies, thereby increasing overall queen production.

From the perspective of a reproductive queen, the optimal investment in daughters practicing the two strategies probably varies with location. Colonies at an expanding edge should experience more reproductive success by investing heavily in claustral daughters that can colonize empty habitat. On the other hand, colonies in the saturated range interior should benefit more from investment in parasitic daughters, as empty habitat is scarce and there are plenty of established colonies with recently deceased queens. Under what we call the *Optimal Investment Hypothesis*, relative investment in claustral versus parasitic queens should evolve as populations expand. In particular, the average investment in claustral queens should increase from the core to the range edge.

We evaluate these hypotheses using an agent-based computer model to track dispersal and colony founding in expanding fire ant populations over 25 years. To examine the ecological effects of reproduction-dispersal polymorphisms, we compare demography, habitat occupancy, and range expansion among populations differing in relative investment in claustral versus parasitic queens. To examine fitness implications of the two strategies, we monitor changes in relative investment within a single variable population as it expands. While we focus on the dynamics of range shifts, our results also provide insight into factors shaping the evolution of reproductive strategies in ants.

Methods

Model design

We constructed an agent-based model in the program R (R Core Team 2012), which simulated the behavior of individual queens and colonies, and allowed us to examine properties of populations of interacting individuals. The inputs to our model determined colony growth and death, competitive territory growth, and reproduction and dispersal through the production of new queens. With these first principles in place, we seeded hypothetical arenas with colonies possessing specified combinations of traits, and monitored how the populations behaved over time (Figure 20).

Each simulation began with 50 colonies distributed randomly over 900 square meters within an arena (Figure 20A). The arena was 50 meters wide, bounded on its lower side, and unbounded in the upper direction. Starting colonies were assigned a colony size of 1,000 workers, approximating that of a young colony around 6 months old (Markin et al. 1973), and began with square territories measuring 3 by 3 meters. The vital parameters of all colonies (age, size, parent lineage, etc.), as well as their territory sizes and shapes, were maintained in a spatial polygons data frame (Pebesma & Bivand 2005; Bivand et al. 2013) throughout the simulation. We then simulated the growth, death, reproduction and dispersal of the colonies at monthly intervals for 25 years (300 time steps, ~4-8 generations, Tschinkel 2013). At each time step, the model progressed through a series of calculations outlined below.

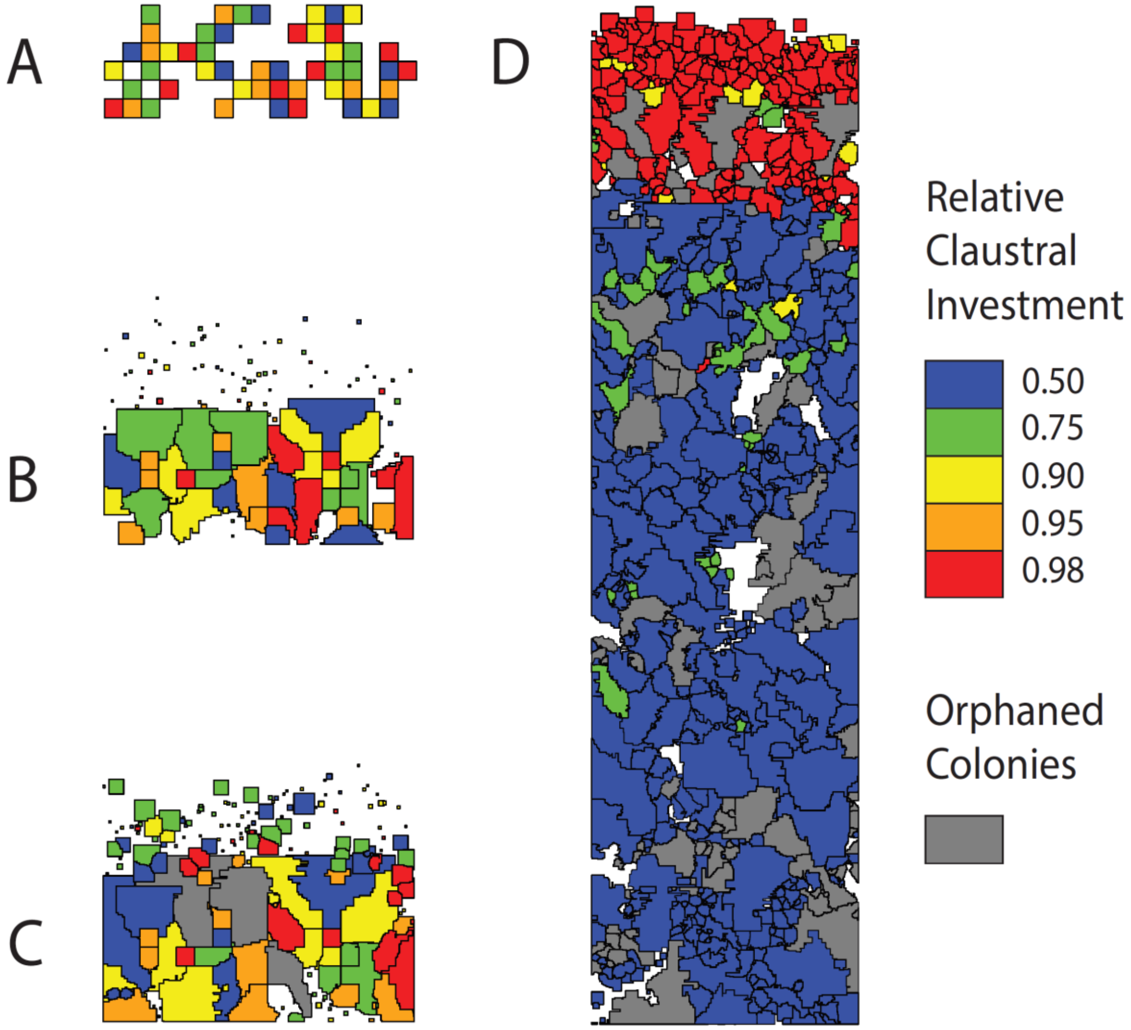

Figure 20. Example simulation of a mixed population consisting of several lineages after A) 0 months, showing starting conditions; B) 22 months, after their first season of dispersal; C) 34 months, showing orphaned colonies (gray); and D) 300 months, at the end of the simulation. Simulation arenas are 50 meters wide. Colors represent lineages that invest different amounts of effort in claustral versus parasitic queens.

Step 1: Colony mortality

Every month we calculated a survival probability for each colony based on its age and size (number of workers). Survival probability was determined from a combination of two functions. The first was an asymptotic function for monthly survival based on colony size:

$$P_{\text{pop}} = 1 - e^{-0.002 * (\text{number of workers} + 1{,}250)}$$

This size-based function stipulated that smaller colonies were more likely to die, as they are more vulnerable to competitive interactions and demographic and environmental variability (Tschinkel 2013). The second function was based on the maximum longevity of queens and their limited supply of stored sperm, derived from a single mating early in life. This function was a flat power function that maintained a high but decreasing probability of survival before rapidly reducing to zero at about six years old (Tschinkel 1987):

$$P_{\text{age}} = -2/10^{30} * (\text{age in months})^{16} + 1$$

The final survival probability was the product of the two functions P_{pop} and P_{age}. We applied this probability to each colony stochastically by comparing the calculated probability to a random value between 0 and 1. Colonies with probabilities greater than the randomly generated number survived. Colonies with probabilities lower than the randomly generated number lost their reproductive queen, became orphaned colonies, and began declining in size as the remaining workers died off (see *Step 2: Colony growth*).

Step 2: Colony growth

Every month we calculated the number of workers in each colony using a conditional function that applied different equations based on the current colony size and queen condition. Newly established colonies started with 40 workers, approximating the size of colonies just over one month old (Markin et al. 1973; Porter & Tschinkel 1986). In subsequent time steps we calculated the potential for colony growth using one of two equations. For small colonies with less than 2,500 workers we calculated growth according to a simple exponential model:

$$P_{\text{init}} = 8.41 * e^{0.972 * (t + 1)}$$

where t = current time in months (fit to data in Tschinkel 1988a and Booth & Dhami 2008). For larger colonies of at least 2,500 workers we used a logistic growth function (adapted from Tschinkel 1993b):

$$P_{\text{init}} = 165{,}000/(1 + 83 * e^{-1.26/12 * (t + 1)})$$

where t = current time in months. Fire ant colony sizes fluctuate seasonally, such that they are at their largest in January and smallest in summer. To incorporate these seasonal oscillations, we augmented the colony growth equations with a cosine function (adapted from Tschinkel 1993b):

$$f = \cos(2 * \text{pi} * (month - 1)/12) * 55{,}000$$

where *month* equals the current calendar month (1 to 12). Large colonies experience larger size fluctuations than do small colonies (Tschinkel 1993b), so we adjusted the oscillations using a weighting factor (derived from Tschinkel 1993b, by substituting a size-based function for the time-based term in the weighting factor on page 431):

$$g = 1{,}388{,}407 * (\text{number of workers})^{-1.1678667}$$

The fluctuation for any given colony equaled the oscillation function f divided by the weighting factor g. We combined all the growth functions as follows to calculate the total potential colony size in any given time step:

$$P_{\text{pot}} = P_{\text{init}} + f/g$$

The colony's new size was then provisionally updated in the spatial polygons data frame. A colony's growth is related to its territory size, however, which determines the resources available to it (Tschinkel et al. 1995). We therefore adjusted potential colony growth in cases where a colony's territory size was limiting (see *Step 3: Territory growth*).

Orphaned colonies whose queens had died (see *Step 1: Colony mortality*) declined in size instead of growing. We modeled this using a linear function that rendered the colony extinct within six months after queen death:

$$P_t = P_{t-1} - P_{t-1}/(7 - T_d)$$

where P_t is a colony's size at a given time step and T_d equals the number of months since queen death. We deleted orphaned colonies when they dropped below 100 workers in size.

Step 3: Territory growth

Fire ant colonies control exclusive territories from which they harvest the resources needed for growth, maintenance and reproduction, and aggressively defend their territories from neighboring colonies through worker-worker combat (Tschinkel et al. 1995; Tschinkel 2013). The result is a mosaic of irregularly shaped and non-overlapping territories that fill nearly all available habitat (Korzukhin & Porter 1994; Adams 1998). Simulating colony territory growth in a realistic manner was the most complicated element of the model, because we had to address the intrinsic capacity of each colony to expand based on the number of its workers, while accounting for limits on territory growth imposed by neighboring colonies.

The territory growth process began by reducing the primary spatial polygons data frame—effectively, the map of all colonies—to a subset of colonies that were capable of growth. This subset excluded all colonies that were completely enclosed by neighboring colonies as well as those whose colony sizes were insufficient to warrant territory expansion beyond their current extents. To determine whether a colony was large enough to grow its territory, we calculated the expected number of workers for the current territory size based on the density of workers per area in mature colonies (1 worker per 6.4 cm^2, Tschinkel et al. 1995). If a colony's size (calculated in *Step 2: Colony growth*) exceeded that expected from its territory size, then we included it among those slated for territory expansion.

For each territory to be expanded, we then used the *gBuffer* function in the *rgeos* package (Bivand & Rundel 2015) to draw a buffer around the territory. The width of the buffer was determined by the territory size of the colony, such that larger territories were assigned larger buffers:

$$\text{buffer width} = (\text{colony area})^{0.5} * 0.2$$

After establishing a buffer area around each colony territory, we used the *gDifference* function in the *rgeos* package (Bivand & Rundel 2015) to subtract from the buffers all areas occupied by existing colonies and areas beyond the arena boundaries. The remaining polygons outlined the areas into which territory growth was possible, and were added to the colony's current territory. In many cases, however, the potential growth areas of different colonies overlapped. When this occurred we overlaid a 4 x 4 grid on the overlapping area, dividing it into subpolygons. We then assigned equal numbers of grid cells to the two parent territories. We did this in a manner that ensured that grid cells were not separated from the parent territories to which they were assigned. Overlapping potential growth areas were thus divided between two parent colonies roughly equally. This means of allocating space applied only to pairwise overlaps. In cases where potential growth polygons from three or more colonies overlapped, we first divvied up the overlap associated with the two oldest colonies from the group and then assigned the remaining area to the other parent colonies. In cases where large colony territories grew to completely surround smaller ones, the subsumed colony was deleted and the larger one allowed to expand into the vacated area, reflecting the competitive dominance of large colonies and their intolerance of smaller colonies within their borders (Tschinkel 2013).

Once all territory sizes were adjusted, we again calculated the expected colony size based on territory size (1 worker per 6.4 cm^2, Tschinkel et al. 1995), and compared this value with the current colony size (see *Step 2: Colony growth*). The lesser of the two values was retained as the colony size for the current time step. In this way colony and territory growth interacted in a mutual feedback, such that potential colony growth at each time step determined the potential for territory expansion, and realized territory expansion allowed or limited realized colony growth. This feedback captures the situation in the field, in which the number of workers in a colony determines its competitive ability, but a colony's territory size determines the resources available for the production of workers (Tschinkel 2013). Colony territories do not shrink when the number of workers declines due to seasonal fluctuations (Tschinkel et al. 1995). Territory sizes in our model therefore remained static during seasonal worker declines or following the death of queens (see *Step 2: Colony growth*).

Simultaneously expanding individual territories in a manner that fills available space but does not allow overlap was difficult, largely due to the frequent generation of topographies that were invalid or incompatible with the spatial functions we employed.

Invalid topographies often included self-intersecting polygons or complex polygons that closed into lines upon territory expansion. To avoid some of these errors, we rounded polygon coordinates to the nearest millimeter. We alleviated the vast majority of errors that still occurred by using the *clgeo_Clean* function from the *cleangeo* package (Blondel 2015), which corrected problematic polygons, with negligible changes to their areas and outlines. Rare topology errors, however, still arose that could not be handled. In these instances we determined which of the polygons in the spatial polygons object let to the invalid topography and deleted it. These errors occurred only up to 10 times for each 300-month simulation involving over 10,000 colonies, and we do not think they compromised our results. When the previous measures failed to avoid invalid topographies, we implemented a routine that essentially went back in time in the simulation to January of the preceding year (12 to 23 months before the error was encountered). When this happened, all model parameters reverted to a state stored in memory corresponding to that particular January. The simulation then proceeded forward, and due to the stochasticity in the model was unlikely to encounter the same error. The end result was a model structure that could complete a 300-month simulation about 95% of the time.

Step 4: Reproduction and dispersal

Once fire ant colonies grow sufficiently large, they begin producing queens to disperse and reproduce. In our model, colonies started to produce queens after growing to 30,000 workers in size (Markin et al. 1973; Vargo 1988). For each colony for each year, we determined queen production based on its size in January, which is an indicator of the energy reserves available for reproduction (Tschinkel 1993b). Colonies with fewer than 50,000 workers in January produced enough queens to weigh 16% as much as the colony's combined worker biomass, whereas larger colonies produced enough to equal 35% of worker biomass (Tschinkel 1993b). Determining worker biomass from the number of workers is not straightforward, as larger colonies produce larger individual workers. We thus calculated total worker biomass nonlinearly:

$$\text{worker biomass} = 0.086 * P^{1.178}$$

where *worker biomass* is dry mass measured in milligrams and P equals the number of workers in the colony (derived from Figure 23 in Tschinkel 1993b).

To translate total queen biomass into individual queens, we first determined the relative investment in claustral versus parasitic queens. We did this using a variable, *ptype*, which could range from ~0 to 1 and represented the proportion of queen biomass invested in claustral queens. The value of *ptype* was assigned to the initial colonies in each simulation, was inherited by daughter colonies thereafter, and varied across simulations (see *Experimental design*). We divided the total mass of each queen type by average queen mass to determine the total number of queens to produce that year. For this step we assumed an average dry mass of 4.7 mg for parasitic queens and 7.2 mg for claustral queens (Helms & Godfrey 2016). Finally, to simulate the near total mortality that occurs during mating and early colony founding, we randomly deleted 95% of the queens of both types produced by each colony. Mortality among dispersing fire ant queens is known to be high (estimated at over 99%, Whitcomb et al. 1973; Tschinkel 1992) but is difficult to measure. Our value of 95% is therefore just an estimate. The simulation results were not sensitive to this random queen mortality, however, as the arenas were nearly fully occupied by territories, and competition for space appeared to be the dominant driver of colony growth (see *Results*). The remaining queens from all colonies were added to a data frame that recorded their type and colony of origin.

Although annual queen production was determined in January, queen dispersal took place over several months throughout the year. For parasitic queens, half the queens dispersed in February and half in March (Morrill 1974; Tschinkel 1996). Claustral queens dispersed in the spring and summer—20% in April, 20% in May, 30% in June, 20% in July, and 10% in August (Morrill 1974). Queens dispersed away from their parent colony according to random draws of direction and distance. Direction was drawn from a uniform distribution of 0 to 360 degrees. For claustral queens distance was drawn from a gamma distribution with a mean of 20 meters and a standard deviation of 2 meters. For parasitic queens we used a gamma distribution with a higher mean and standard deviation (30 ±3 meters), to reflect the more favorable flight morphology that results from their reduced weight (Helms & Kaspari 2014, 2015, 2016). Fire ant queens in the field routinely disperse several hundred meters and occasionally several kilometers (Tschinkel 2013). Our dispersal distances are thus not realistic. Attempts to use more realistic dispersal distances resulted in extremely large numbers of colonies that caused simulations to progress slowly and eventually crash. Based on these trials, however, simulations with long dispersal distances appeared to have the same dynamics as those with short distances, with the exception that farther dispersal causes populations to expand their ranges more rapidly. Queens that dispersed across the lower side of the arena, the only bounded edge, were considered dead and we deleted them. Those that flew across the right or left edges of the arena were repositioned within the arena but on the opposite side, as if the left-right dimension was continuous and circular (*i.e.*, a cylinder). There were no constraints on upward dispersal.

Survival and successful colony founding depended on where a queen landed. Claustral queens that landed within an area occupied by any existing colony were killed. Those that landed on unoccupied space, however, were transformed in the next time step into newly established colonies, each with 40 workers and a territory of 0.1 m^2 (see *Step 2: Colony growth*, Figure 20B). Parasitic queens, on the other hand, survived only if they landed within the territory of an orphaned colony (see *Step 1: Colony mortality*, Figure 20C) and were killed if they landed in unoccupied space or the territory of a colony whose queen was alive. If two or more claustral queens landed in unoccupied space close enough for their initial territories to overlap, or if multiple parasitic queens landed within the same orphaned colony's territory, a single winning queen was chosen at random and the remaining queens killed. Orphaned colonies that received a parasitic queen retained their current colony and territory size, were no longer considered to be orphaned, and began to grow again (see *Step 2: Colony growth*). They were, however, assigned new parameters for parent lineage, age, and *ptype* matching those of the new queen.

Step 5: Advancing to the next time step

After any newly established colonies were added to the spatial polygons data frame, we performed error checking steps to ensure that the topology of the new set of polygons was acceptable (see *Step 3: Territory growth*). The updated data frame was then saved, and we advanced counters that kept track of the time step and calendar month. The entire process was then repeated for the next time step.

Experimental design

Using the model described above, we ran two sets of simulations, the first to examine the effects of reproductive polymorphisms on populations, and the second to examine the fitness implications for colonies investing in the two reproductive strategies. For the first set of simulations, we seeded arenas with 50 colonies that all invested the same amount of effort in claustral versus parasitic queens (*i.e.*, they had the same *ptype* value). We then ran each simulation for 300 months (25 years), starting in January. Each simulation represented one of six treatments, wherein populations had relative claustral investment set to 1, 0.98, 0.95, 0.90, 0.75, or 0.50. We ran 72 simulations for each treatment using a C4.8xlarge virtual computer available through Amazon Web Services, which allowed us to run 36 simulations at a time. After accounting for failed simulations, we ended up with 67 to 69 replicates of each treatment for a total of 407 simulations (n = 69 at *ptype* = 1; n = 68 at 0.98, 0.95, and 0.90; and n = 67 at 0.75 and 0.5). We then compared demography, habitat occupancy, and range expansion among the populations that emerged from the six treatments after 300 months.

For each simulation in this first set, we measured the average colony size, average territory size, percentage of available area occupied by all colonies, percentage of colonies headed by parasitic queens, and the maximum upward extent of the range. The upward extent was defined by the maximum y-coordinate among all the territory outlines. To examine spatial patterns we divided the occupied area into sampling windows that were 5 meters high in the up-down axis and extended across the 50-meter width of the arena. We focused on colony size rather than age, because in fire ants (and other social insects) a colony's size is a better indicator of its ecological impact and reproductive potential. Moreover, a colony's size at any age can vary over orders of magnitude due to environmental factors and competitive interactions (Tschinkel 2013).

The second set of simulations investigated fitness and optimal investment of colonies producing the two queen types. For these simulations, we seeded each arena with 50 colonies varying in *ptype* value. Each of five *ptype* values—0.98, 0.95, 0.90, 0.75, and 0.5—was represented by 10 starting colonies, yielding an initial average claustral investment of 0.847. We then ran the simulation for 300 months (25 years), starting in January, allowing average claustral investment to evolve through the differential survival and reproduction of colonies with different *ptype* values (Figure 20D). We ran 72 simulations using the virtual computer described above, resulting in 66 completed replicates. At the end of the simulation we measured the average claustral investment among colonies large enough to reproduce (≥30,000 workers, see *Step 4: Reproduction and dispersal*) in 5 x 50 meter sampling windows.

Results

Population effects

Simulated colony size and territory distributions matched those observed in the field, such that populations consisted of many small colonies and few large ones (Tschinkel 2013), with territories closely packed and irregularly shaped (Adams 1998, Figure 20). As predicted by the Immortality Hypothesis, investing in parasitic queens increased average colony size by 23 to 92% over populations producing only claustral queens (ANOVA $F_{5,\,401} = 723.4$, $P = 2 \times 10^{-16}$, Figure 21A). Every decrease in claustral investment below 0.98 increased average colony size in the population (Tukey's post-hoc tests, 1 to 0.98 comparison $P = 0.997$; all other Ps $< 10^{-7}$), from a low of 9,306 workers per colony at total claustral investment to 17,877 workers per colony at half claustral investment. The same results occur when comparing colony territory sizes (ANOVA $F_{5, 401} = 850.5$, $P = 2 \times 10^{-16}$, Figure 21B). Mean territory size in the population increased by up to 133% over populations producing only claustral queens. Every increase in parasitic investment increased average territory size (Tukey's post-hoc tests, all Ps $< 10^{-7}$), from a low of 7.2 m^2 at total claustral investment to 16.8 m^2 at half investment. Even a 2% decrease in claustral investment, from 1 to 0.98, caused a 15% increase in average territory size to 8.26 m^2.

Also as predicted, fire ant colonies occupied up to 12% more of the available habitat in populations that produced parasitic queens (Figure 22A). In all populations habitat occupancy fluctuated around consistently high values before dropping to zero at the expanding range margin. But fluctuations were dampened and habitat occupancy was usually higher in populations producing parasitic queens. Mean habitat occupancy over the whole range varied from 75.3% (±2.57) in populations that produced only claustral queens to 84.5% (±2.68) in those that invested half their effort in parasitic queens. These values correspond well with rough field estimates of fire ant territory coverage of available habitat (>90%, Korzukhin & Porter 1994).

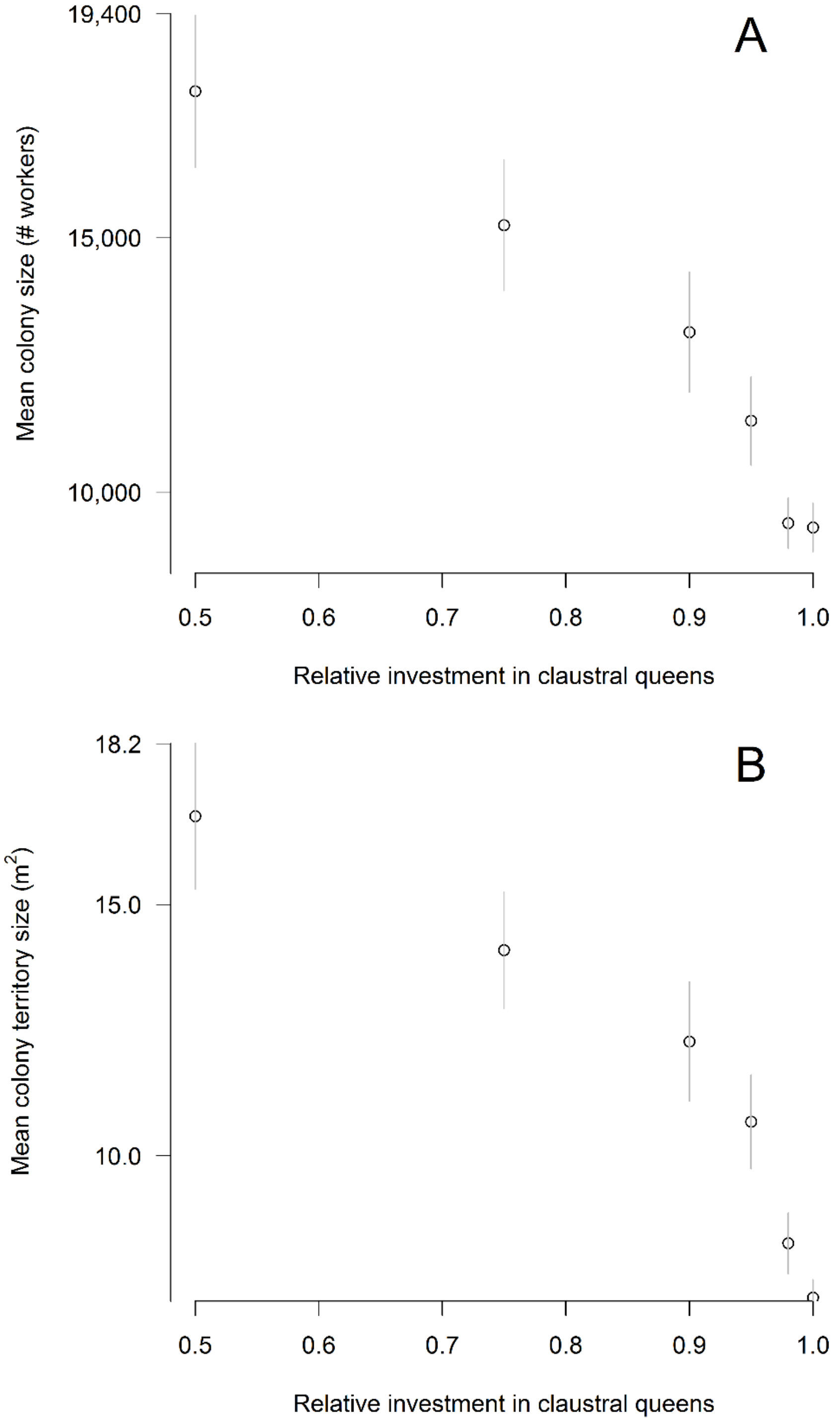

Figure 21. Because parasitic queens extend the ecological lifespan of colonies, populations that invest more in parasitic queens experience larger average colony sizes (A) and colony territory areas (B). Points show means over all simulations for a given reproductive investment, and error bars show standard deviations. In (A), all values differ (*P* < 0.001) except for those at 1 and 0.98 relative investment (*P* = 0.997); in (B) all values differ (*P* < 0.001).

The observed changes in demography and habitat occupancy were driven by the parasitic takeover of orphaned colonies. Even a slight increase in the production of parasites, from 0 to 2% of reproductive investment, led to an average of 43.1% (±20.2%) of colonies being headed by parasitic queens (Figure 22B). In populations investing a fourth to a half of their effort in parasites, there were regions where nearly 100% of colonies were headed by parasitic queens (range-wide average of 75.4 ±31.6% for 0.75 claustral investment, 74.8 ±33.7% for 0.5 claustral investment).

Despite its positive effects on average colony size and persistence, investment in parasitic queens decreased the rate of range expansion by up to 4% (ANOVA $F_{5, 401}$ = 43.593, $P = 2 \times 10^{-16}$, Figure 23), from an average maximum of 196.2 (±3.1) meters per simulation in totally claustral populations to 188.8 (±3.2) meters in populations investing half their effort in parasitic queens. Decreasing investment in claustral queens from 1 to 0.9 had no effect (Tukey's post-hoc tests, $P > 0.137$), but further decreases to 0.75 or 0.5 slowed range expansion ($P < 0.003$). Parasites thus appear to affect range expansion primarily by slowing it down through the diversion of investment from claustral queens that can colonize vacant sites, rather than speeding it up by stabilizing larger, more productive colonies.

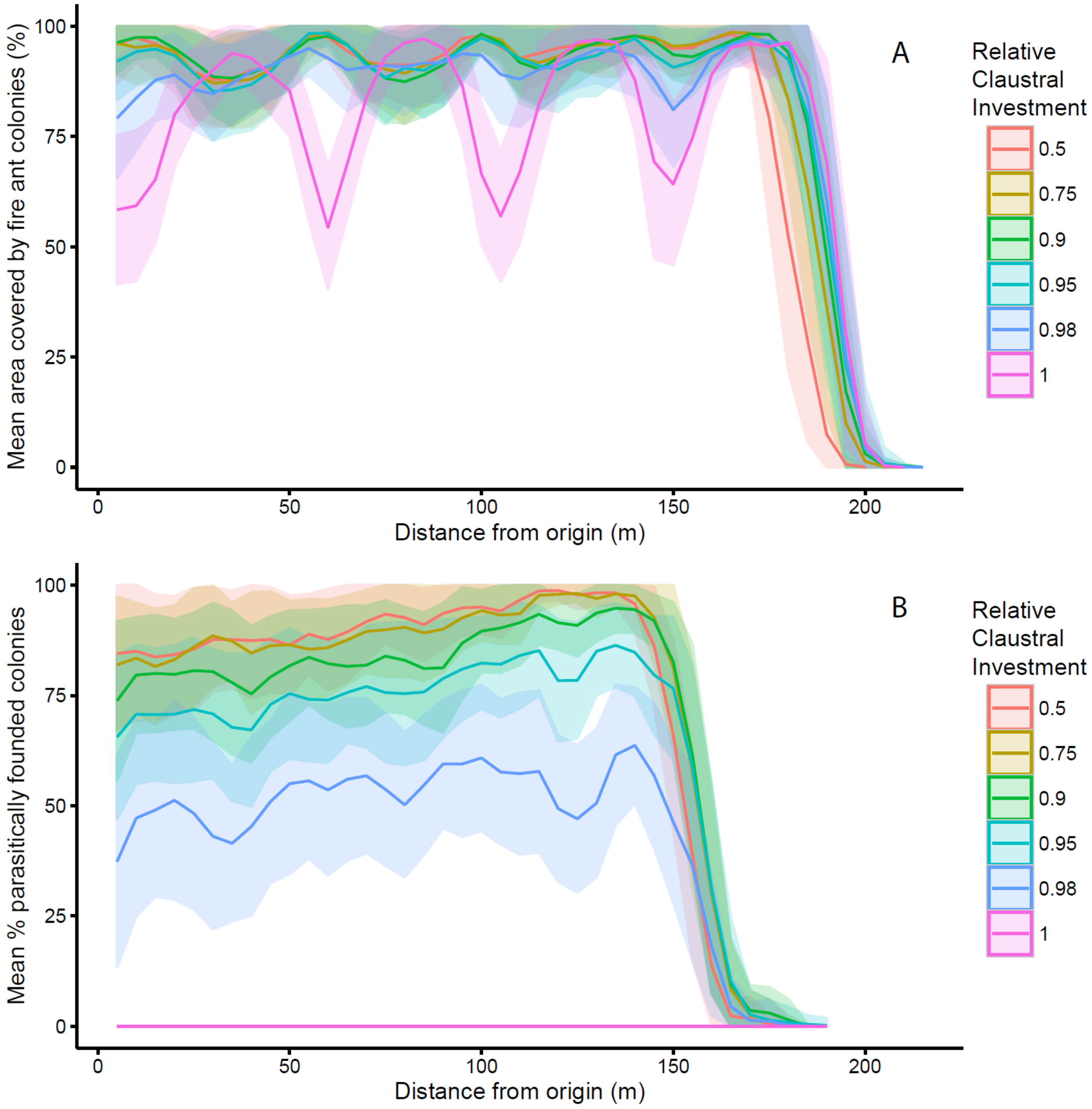

Figure 22. (A) Percentage of available habitat occupied by fire ant colonies versus distance from the origin (bottom) of a range. Investment in parasitic queens increases and stabilizes the amount of habitat occupied by fire ant colonies. (B) The percentage of all colonies that are headed by a parasitic queen versus distance from the origin of a range. Even small investments in parasitic queens lead to high proportions of parasitically founded colonies in the range interior. In all simulations, only claustrally founded colonies occur at the extreme range edge. Colors denote different levels of reproductive investment, lines show averages over all simulations for a given investment, and shading shows standard deviations.

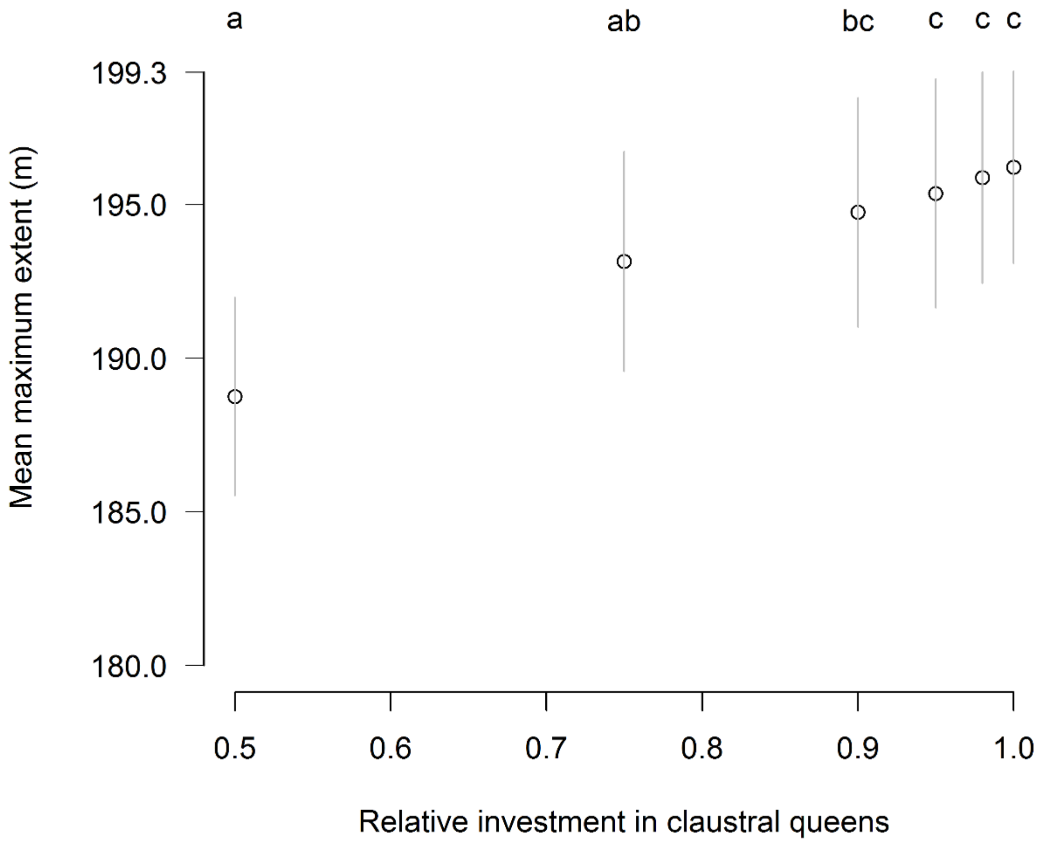

Figure 23. Investment in parasitic queens slows range expansion by diverting resources from the production of claustral queens. Bar heights show mean maximum extents of spreading populations over all simulations for a given reproductive investment, and error bars show standard deviations. Bars with different letters differ at $P < 0.003$.

Optimal investment

Mature colonies occurred at an average density of 323 ±119 colonies per hectare (n = 66), which is strikingly similar to field estimates from monogyne populations in the southern USA (300 ±240 colonies/ha, Porter et al. 1991). Core and edge populations experienced divergent selection regimes during range expansion. As predicted by the Optimal Investment Hypothesis, a pattern emerged over the course of every simulation wherein colonies in the range interior invested more heavily in parasitic queens and less in claustral queens (Figure 24). The innermost populations averaged slightly above

50% investment in claustral queens (minimum 0.51 ±0.028), which was the minimum allowed in our simulation. At the same time, edge populations retained a heavy investment in claustral queens, with average values approaching 100% (maximum claustral investment 0.96 ±0.015). In these simulations, expansion-related selection has created a geographic gradient in life history strategy within a single variable species.

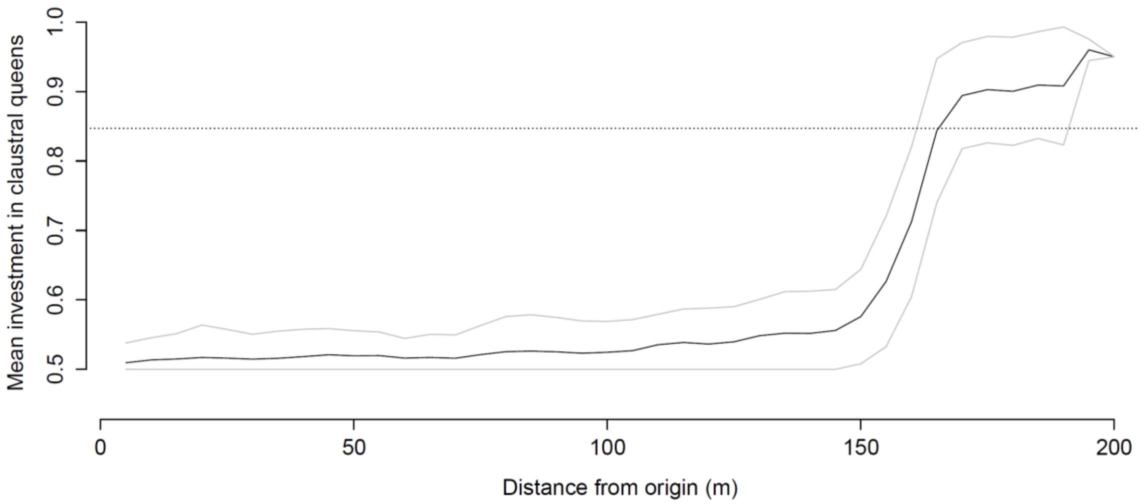

Figure 24. Mean reproductive investment of mature colonies from the range origin (bottom) to the top edge. Gray lines show standard deviations, dashed line shows starting average of 0.847. Populations in the saturated range interior evolve greater investment in parasitic queens, while those at the uninhabited range edge retain greater investment in dispersing claustral queens.

Discussion

Range expansion is a defining character of invasive ants. In species practicing alternate life histories, range dynamics are likely affected by relative investment in different strategies. In our simulations of red imported fire ants, the production of parasitic queens results in larger average colony and territory sizes and higher habitat occupancy. On the other hand, by diverting investment from claustral queens that can colonize vacant habitats, the production of parasitic queens may slow range expansion. Range expansion in turn affects the fitness of colonies producing the two queen types.

Colonies at expanding range edges benefit more by investing in claustral queens that can colonize the surrounding vacant habitat, whereas those in the crowded range interior profit from investing more in parasitic queens that can take over orphaned colonies. Divergent selection regimes appear to drive the evolution of different levels of reproductive investment based on their distance from the range edge.

The effects of range expansion also shed light on other factors shaping the evolution of reproductive strategies in ants. Parasitic founding is thought to be more beneficial in stable saturated environments, and claustral founding to be more beneficial in vacant or disturbed habitats (DeHeer & Tschinkel 1998; Tschinkel 2013). The evolved population differences in our simulations support this notion and also parallel differences among co-occurring fire ant species in the field. Along the US Gulf Coast, *Solenopsis invicta* lives alongside the closely related tropical fire ant, *Solenopsis geminata*, which has a similar life cycle (McInnes & Tschinkel 1995). Within this range, the introduced *S. invicta* occurs primarily in highly disturbed anthropogenic habitats, while the native *S. geminata* occupies more stable natural habitats (Tschinkel 1988b). These habitat differences are mirrored by reproductive differences, with *S. geminata* investing three to four times as much effort in parasitic queens than *S. invicta* (33% of investment versus <10%). Similarly, our results suggest that within a species older populations should evolve a more parasitic, less dispersive, lifestyle than recently established ones.

Our simulated populations generally behaved realistically, highlighting the model's value for investigating fire ant ecology. Our populations displayed near total occupancy of available habitat (Korzukhin & Porter 1994), closely packed irregularly shaped territories (Adams 1998), size distributions consisting of many small colonies and a few large ones (Tschinkel 2013), and population densities similar to those in the field (323 ±119 colonies/ha simulated versus 300 ±240 in the field, Porter et al. 1991). We note, on the other hand, that in our simulated populations, the observed frequency of parasitic founding and the optimal reproductive investment in interior colonies (>40% of colonies headed by parasites, 40–50% investment in parasitic queens) more accurately describe the native *S. geminata* (35% of colonies, 33% investment in parasites, McInnes & Tschinkel 1995) than *S. invicta* (3.5% of colonies, <10% investment in parasites, DeHeer & Tschinkel 1998). Our goal is not to make absolute predictions about fire ant biology, however, but rather to investigate the interplay between reproductive strategy and range dynamics within a given species.

We made several simplifying assumptions in constructing our model. We assumed, for example, that habitat is constant and homogeneous and that lineages do not interbreed. Incorporating disturbance—to better capture the ecological preferences of *S. invicta*—would shift optimal investment toward more claustral queens by providing a steady supply of vacant habitat in which to found colonies. Allowing gene flow among lineages would slow divergence between interior and edge populations, probably shifting investment toward more claustral queens in the interior. Furthermore, a substantial minority of fire ant populations in the field (≤20%, Porter et al. 1997) are polygyne and practice fundamentally different life histories in which colonies contain many unrelated queens and reproduce vegetatively by budding or splitting (Tschinkel 2013). Finally, introduced populations of *S. invicta* compete with (Porter et al. 1988; Tschinkel 1988b) or hybridize with (Ometto et al. 2012) other fire ant species, creating a complex network of interspecific interactions affecting dispersal, colony growth, and reproductive success. A complete model of fire ant invasions would incorporate all these variants, and is beyond the scope of our current study.

The rapid spread of several invasive ant species around the globe, through multiple introduction events, provides a valuable opportunity to investigate the interplay between range expansion, dispersal, and reproduction. Because small differences in reproductive strategy cause pervasive changes in demography, habitat occupancy, range expansion, and the response to expansion-related selection, founder effects may play a major role in determining the ecological impacts of introduced ants. Subsequent selection associated with rapid range expansion may further shape the evolution of introduced populations. For similar reasons, some native ant species may be unable to shift their ranges rapidly enough to track climatic changes, and those that do may experience changes in dispersal ability or reproductive ecology as a result. In a world where ant range shifts are increasingly likely (Colwell et al. 2008), predicting these outcomes has substantial practical importance. Agent-based models are a useful approach for addressing these issues, given sufficiently detailed life history inputs, and provide a relatively rapid and low-cost method of examining future scenarios.

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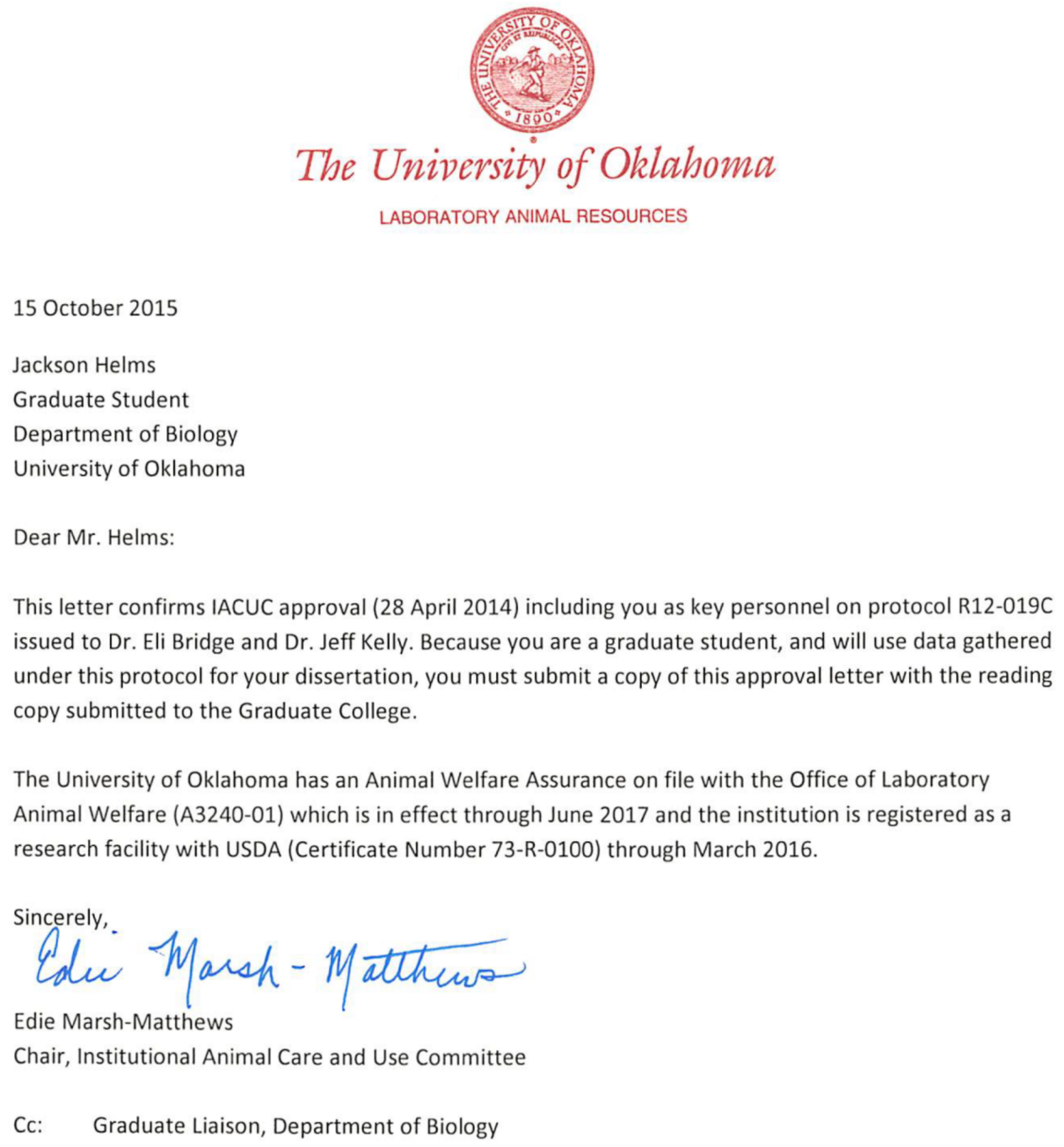
Appendix: IACUC Approval Letter



The University of Oklahoma
LABORATORY ANIMAL RESOURCES

15 October 2015

Jackson Helms
Graduate Student
Department of Biology
University of Oklahoma

Dear Mr. Helms:

This letter confirms IACUC approval (28 April 2014) including you as key personnel on protocol R12-019C issued to Dr. Eli Bridge and Dr. Jeff Kelly. Because you are a graduate student, and will use data gathered under this protocol for your dissertation, you must submit a copy of this approval letter with the reading copy submitted to the Graduate College.

The University of Oklahoma has an Animal Welfare Assurance on file with the Office of Laboratory Animal Welfare (A3240-01) which is in effect through June 2017 and the institution is registered as a research facility with USDA (Certificate Number 73-R-0100) through March 2016.

Sincerely,

Edie Marsh-Matthews

Edie Marsh-Matthews
Chair, Institutional Animal Care and Use Committee

Cc: Graduate Liaison, Department of Biology