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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.

Key words: Alates, *Azteca instabilis*, colony founding, dispersal, Formicidae, Found or Fly hypothesis, mating flight, nutrient investment, reproductive strategy, tradeoffs.

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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 & 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 1993, 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 1988, 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 & al. 1976, VOSS & BLUM 1987, KELLER & PASSERA 1989, KELLER & ROSS 1993a, DEHEER & 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 & al. 1985, KELLER & PASSERA 1989, MARTINEZ & WHEELER 1994, HAHN & 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 1993, LIU & 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 & al.1995, VOGT & al. 2000, WIERNASZ & COLE 2003), dispersal distance (FORTELIUS & al. 1987, SUNDSTRÖM 1995, ZERA & DENNO 1997, RÜPPELL & al. 1998, LACHAUD & al. 1999, GU & 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 & 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 & al. 1998, RÜPPELL & HEINZE 1999, LACHAUD & al. 1999, HEINZE & KELLER 2000, PEETERS & ITO 2001, STEINER & al. 2006, PEETERS 2012, PEETERS & 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 & al. 2001a, KASPARI & al. 2001b), 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 1993, 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., 3 m and 27 m above ground level (KASPARI & 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 & 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 & 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 & 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 & 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 & 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 & al. 2000, DARVEAU & al. 2005). To calculate wing loading, body mass was divided by the total area of all four wings and converted to Nm^{-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 two thirds.

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 × 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.

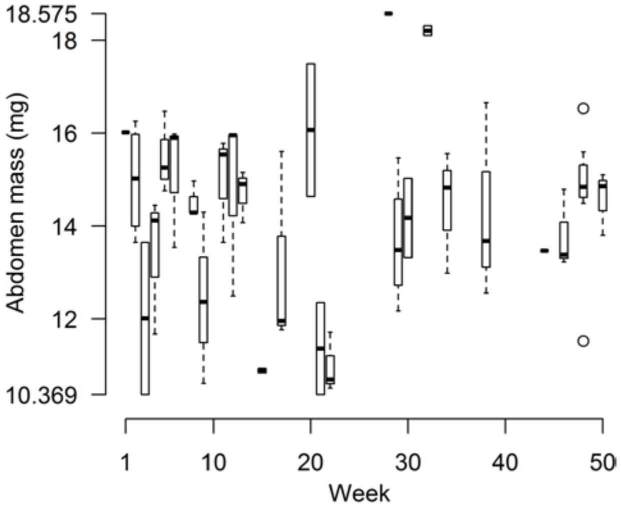

Fig. 1: Abdomen mass of young queens varies weekly throughout the year (n = 73, Kruskal-Wallis, $P = 0.03$), reflecting the plasticity of this trait. Weeks 1 through 17 correspond to the dry season on Barro Colorado Island. Box plots show medians, quartiles and outliers. Weeks 1, 28 and 44 have only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

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, $P = 0.03$, Fig. 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.

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 ($P < 0.03$, Fig. 2a). Wing loading (mean 2.67 ± 0.23 Nm^{-2}) increased about 40% over the range of abdomen mass ($P < 0.03$, Fig. 2b). For drag reference area (mean 10.11 ± 1.5 mm^2), size-corrected values increased about 2 mm^2 with abdomen mass ($P < 0.03$, Fig. 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.

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 & al. 2005), and be able to fly both faster (VOGT & 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.

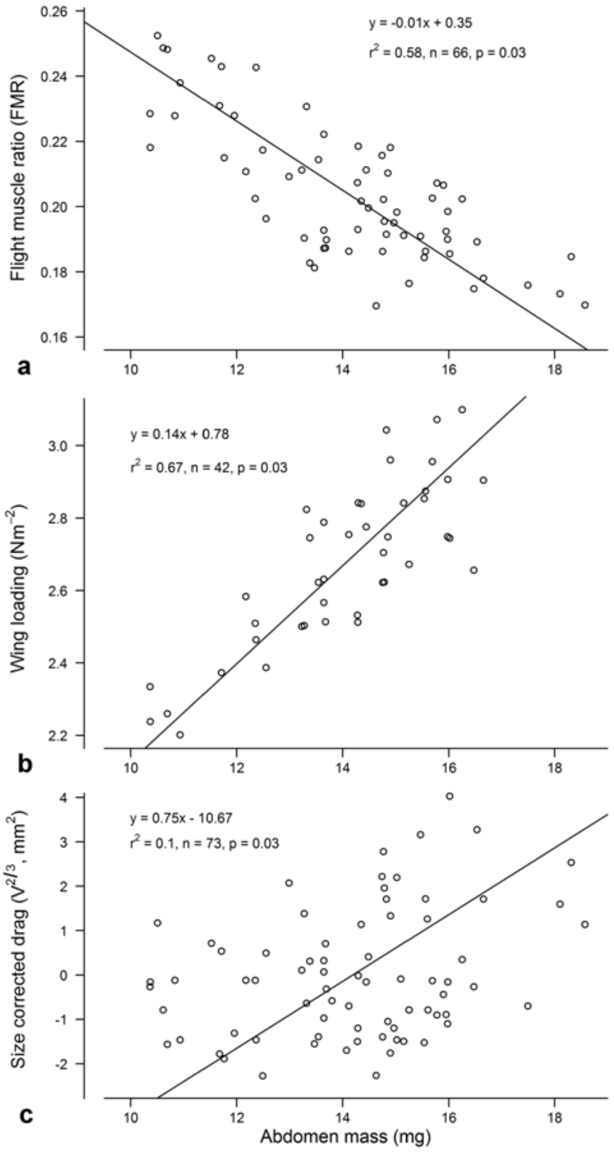

Fig. 2: Nutrient loading adversely impacts flight ability metrics. a) Flight muscle ratio (FMR) declines with nutrient loading. Reduced FMR decreases maneuverability, flight endurance, acceleration and load lifting ability. b) Wing loading increases with nutrient loading. Higher wing loading reduces 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 ordinary least squares regression on head width.

In the fire ant *Solenopsis invicta* a hypothetical difference of 8 mg dry weight, assuming a live to dry weight ratio of 2 (TSCHINKEL 1993), could be expected to produce 65 to 90 more initial workers (TSCHINKEL 1993, 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 six 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 & al. 1976, JUTSUM & QUINLAN 1978, PASSERA & KELLER 1990, PASSERA & al. 1990, VOGT & 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 & al. 1976, PASSERA & KELLER 1990, PASSERA & 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 (J.A. Helms & M. Kaspari, unpubl.). 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, 1993b).

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, 1993b, 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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References

ADAMS, E.S. & BALAS, M.T. 1999: Worker discrimination among queens in newly founded colonies of the fire ant *Solenopsis invicta*. – Behavioral Ecology and Sociobiology 45: 330-338.

ALEXANDER, D.E. 1990: Drag coefficients of swimming animals: effects of using different reference areas. – Biological Bulletin 179: 186-190.

BALAS, M.T. & ADAMS, E.S. 1996: The dissolution of cooperative groups: mechanisms of queen mortality in incipient fire ant colonies. – Behavioral Ecology and Sociobiology 38: 391-399.

BEENAKKERS, A.M.T. 1969: Carbohydrate and fat as a fuel for insect flight. A comparative study. – Journal of Insect Physiology 15: 353-361.

BERNASCONI, G. & KELLER, L. 1996: Reproductive conflicts in cooperative associations of fire ant queens (*Solenopsis invicta*). – Proceedings of the Royal Society B-Biological Sciences 263: 509-513.

BERNASCONI, G. & KELLER, L. 1999: Effect of queen phenotype and social environment on early queen mortality in incipient colonies of the fire ant, *Solenopsis invicta*. – Animal Behaviour 57: 371-377.

BOOMSMA, J.J. & ISAAKS, J.A. 1985: Energy investment and respiration in queens and males of *Lasius niger* (Hymenoptera: Formicidae). – Behavioral Ecology and Sociobiology 18: 19-27.

BROWN, M.J.F. & BONHOEFFER, S. 2003: On the evolution of claustral colony founding in ants. – Evolutionary Ecology Research 5: 305-313.

BRUNA, E.M., IZZO, T.J., INOUYE, B.D., URIARTE, M. & VASCONCELOS, H.L. 2011: Asymmetric dispersal and colonization success of Amazonian plant-ants queens. – Public Library of Science One 6: e22937.

BUSCHINGER, A. & HEINZE, J. 1992: Polymorphism of female reproductives in ants. In: BILLEN, J. (Ed.): Biology and Evolution of Social Insects. – Leuven University Press, Leuven, pp. 11-23.

DARVEAU, C.A., HOCHACHKA, P.W., WELCH, K.C. Jr., ROUBIK, D.W. & SUAREZ, R.K. 2005: Allometric scaling of flight energetics in Panamanian orchid bees: a comparative phylogenetic approach. – The Journal of Experimental Biology 208: 3581-3591.

DAVIDSON, D.W. 1982: Sexual selection in harvester ants (Hymenoptera: Formicidae: *Pogonomyrmex*). – Behavioral Ecology and Sociobiology 10: 245-250.

DEHEER, C.J. 2002: A comparison of the colony-founding potential of queens from single- and multiple-queen colonies of the fire ant *Solenopsis invicta*. – Animal Behaviour 64: 655-661.

DEHEER, C.J., GOODISMAN, M.A.D. & ROSS, K.G. 1999: Queen dispersal strategies in the multiple-queen form of the fire ant *Solenopsis invicta*. – The American Naturalist 153: 660-675.

DILLON, M.E. & DUDLEY, R. 2004: Allometry of maximum vertical force production during hovering flight of neotropical orchid bees (Apidae: Euglossini). – The Journal of Experimental Biology 207: 417-425.

DUDLEY, R. 2000: The biomechanics of insect flight: form, function, evolution. – Princeton University Press, Princeton, NJ, 536 pp.

ELLINGTON, C.P. 1984: The aerodynamics of hovering insect flight. II. Morphological parameters. – Philosophical Transactions of the Royal Society B-Biological Sciences 305: 17-40.

FJERDINGSTAD, E.J. & BOOMSMA, J.J. 1997: Variation in size and sperm content of sexuals in the leafcutter ant *Atta colombica*. – Insectes Sociaux 44: 209-218.

FJERDINGSTAD, E.J. & KELLER, L. 2004: Relationships between phenotype, mating behavior, and fitness of queens in the ant *Lasius niger*. – Evolution 58: 1056-1063.

FORTELIUS, W., PAMILO, P., ROSENGREN, R. & SUNDSTRÖM, L. 1987: Male size dimorphism and alternative reproductive tactics in *Formica exsecta* ants (Hymenoptera: Formicidae). – Annales Zoologici Fennici 24: 45-54.

FOX, J. & WEISBERG, S. 2011: An R companion to applied regression, 2nd edition. – Thousand Oaks, Sage, CA; <http://socserv.socsci.mcmaster.ca/jfox/Books/Companion>, retrieved on 9 July 2013.

GORDON, D.M. & KULIG, A.W. 1996: Founding, foraging, and fighting: colony size and the spatial distribution of harvester ant nests. – Ecology 77: 2393-2409.

GU, H., HUGHES, J. & DORN, S. 2006: Trade-off between mobility and fitness in *Cydia pomonella* L. (Lepidoptera: Tortricidae). – Ecological Entomology 31: 68-74.

GULLAN, P.J. & CRANSTON, P.S. 2010: The insects: an outline of entomology. 4th edition, – Wiley-Blackwell, Chichester, 584 pp.

HAHN, D.A., JOHNSON, R.A., BUCK, N.A. & WHEELER, D.E. 2004: Storage protein content as a functional marker for colony-founding strategies: a comparative study within the harvester ant genus *Pogonomyrmex*. – Physiological and Biochemical Zoology 77: 100-108.

HEDENSTRÖM, A. 1992: Flight performance in relation to fuel load in birds. – Journal of Theoretical Biology 158: 535-537.

HEINZE, J. & KELLER, L. 2000: Alternative reproductive strategies: a queen perspective in ants. – Trends in Ecology & Evolution 15: 508-512.

HEINZE, J. & TSUJI, K. 1995: Ant reproductive strategies. – Researches on Population Ecology 37: 135-149.

HÖLLDOBLER, B. & WILSON, E.O. 1990: The ants. – Belknap Press of Harvard University Press, Cambridge, MA, 732 pp.

HOLM, S. 1979: A simple sequentially rejective multiple test procedure. – Scandinavian Journal of Statistics 6: 65-70.

JOHNSON, R.A. 1998: Foundress survival and brood production in the desert seed-harvester ants *Pogonomyrmex rugosus* and *P. barbatus* (Hymenoptera: Formicidae). – Insectes Sociaux 45: 255-266.

JOHNSON, R.A. 2001: Biogeography and community structure of North American seed-harvester ants. – Annual Review of Entomology 46: 1-29.

JOHNSON, R.A. 2006: Capital and income breeding and the evolution of colony founding strategies in ants. – Insectes Sociaux 53: 316-322.

JUTSUM, A.R. & QUINLAN, R.J. 1978: Flight and substrate utilization in laboratory-reared males of *Atta sexdens*. – Journal of Insect Physiology 24: 821-825.

KASPARI, M., PICKERING, J. & WINDSOR, D. 2001a: The reproductive flight phenology of a neotropical ant assemblage. – Ecological Entomology 26: 245-257.

KASPARI, M., LONGINO, J., PICKERING, J. & WINDSOR, D. 2001b: The phenology of a Neotropical ant assemblage: evidence for continuous and overlapping reproduction. – Behavioral Ecology and Sociobiology 50: 382-390.

KELLER, L. & PASSERA, L. 1988: Energy investment in gynes of the Argentine Ant *Iridomyrmex humilis* (MAYR) in relation to the mode of colony founding in ants (Hymenoptera: Formicidae). – International Journal of Invertebrate Reproduction and Development 13: 31-38.

KELLER, L. & PASSERA, L. 1989: Size and fat content of gynes in relation to the mode of colony founding in ants (Hymenoptera: Formicidae). – Oecologia 80: 236-240.

KELLER, L. & ROSS, K.G. 1993a: Phenotypic basis of reproductive success in a social insect: genetic and social determinants. – Science 260: 1107-1110.

KELLER, L. & ROSS, K.G. 1993b: Phenotypic plasticity and "cultural transmission" of alternative social organizations in the fire ant *Solenopsis invicta*. – Behavioral Ecology and Sociobiology 33: 121-129.

LACHAUD, J., CADENA, A., SCHATZ, B., PEREZ-LACHAUD, G. & IBARRA-NUNEZ, G. 1999: Queen dimorphism and reproductive capacity in the ponerine ant, *Ectatomma ruidum* ROGER. – Oecologia 120: 515-523.

LEGENDRE, P. 2011: Lmodel2: Model II Regression. R package version 1.7-0. – <http://CRAN.R-project.org/package=lmodel2>, retrieved on 9 July 2013.

LEIGH, E.G. Jr., RAND, A.S. & WINDSOR, D.W. 1996: The ecology of a tropical forest. – Smithsonian Press, Washington, DC, 480 pp.

LIU, Z., YAMANE, S., KOJIMA, J., WANG, Q. & TANAKA, S. 2001: Flexibility of first brood production in a claustral ant, *Camponotus japonicus* (Hymenoptera: Formicidae). – Journal of Ethology 19: 87-91.

LONGINO, J.T. 2007: A taxonomic review of the genus *Azteca* (Hymenoptera: Formicidae) in Costa Rica and a global revision of the *aurita* group. – Zootaxa 1491: 1-63.

LONGINO, J.T. 2010: Ants of Costa Rica. – <http://academic.evergreen. edu/ projects/ants/antsofcostarica.html>, retrieved on 21 January 2013.

MARDEN, J.H. 1987: Maximum lift production during takeoff in flying animals. – Journal of Experimental Biology 130: 235-258.

MARDEN, J.H. 2000: Variability in the size, composition, and function of insect flight muscles. – Annual Review of Entomology 62: 157-178.

MARKIN, G.P., DILLIER, J.H., HILL, S.O., BLUM, M.S. & HERMANN, H.R. 1971: Nuptial flight and flight ranges of the imported fire ant, *Solenopsis saevissima richteri* (Hymenoptera: Formicidae). – Journal of the Georgia Entomological Society 6: 145-156.

MARTINEZ, T. & WHEELER, D.E. 1994: Storage proteins in adult ants (*Camponotus festinatus*): roles in colony founding by queens and in larval rearing by workers. – Journal of Insect Physiology 40: 723-729.

MCARDLE, B.H. 1988: The structural relationship: regression in biology. – Canadian Journal of Zoology 66: 2329-2339.

MCINNES, D.A. & TSCHINKEL, W.R. 1995: Queen dimorphism and reproductive strategies in the fire ant *Solenopsis geminata* (Hymenoptera: Formicidae). – Behavioral Ecology and Sociobiology 36: 367-375.

MERCKX, T. & VAN DYCK, H. 2006: Landscape structure and phenotypic plasticity in flight morphology in the butterfly *Pararge aegeria*. – Oikos 113: 226-232.

MINTZER, A.C. 1987: Primary polygyny in the ant *Atta texana*: number and weight of females and colony foundation success in the laboratory. – Insectes Sociaux 34: 108-117.

NICHOLS, B.J. & SITES, R.W. 1991: Ant predators of founder queens of *Solenopsis invicta* (Hymenoptera: Formicidae) in Central Texas. – Environmental Entomology 20: 1024-1029.

NIELSEN, M.G., SKYBERG, N. & PEAKIN, G. 1985: Respiration in the sexuals of the ant *Lasius flavus*. – Physiological Entomology 10: 199-204.

NONACS, P. 1992: Queen condition and alate density affect pleometrosis in the ant *Lasius pallitarsis*. – Insectes Sociaux 39: 3-13.

NORBERG, U. & LEIMAR, O. 2002: Spatial and temporal variation in flight morphology in the butterfly *Melitaea cinxia* (Lepidoptera: Nymphalidae). – Biological Journal of the Linnean Society 77: 445-453.

NORBERG, U.M. & RAYNER, J.M.V. 1987: Ecological morphology and flight in bats (Mammalia: Chiroptera): wing adaptations, flight performance, foraging strategy and echolocation. – Philosophical Transactions of the Royal Society B-Biological Sciences 316: 335-427.

ODE, P.J. & RISSING, S.W. 2002: Resource abundance and sex allocation by queen and workers in the harvester ant, *Messor pergandei*. – Behavioral Ecology and Sociobiology 51: 548-556.

PASSERA, L. & KELLER, L. 1990: Loss of mating flight and shift in the pattern of carbohydrate storage in sexuals of ants (Hymenoptera: Formicidae). – Journal of Comparative Physiology B 160: 207-211.

PASSERA, L., KELLER, L., GRIMAL, A., CHAUTEMS, D. CHERIX, D., FLETCHER, D.J.C., FORTELIUS, W., ROSENGREN, R. & VARGO, E.L. 1990: Carbohydrates as energy source during the flight of sexuals of the ant *Formica lugubris* (Hymenoptera: Formicidae). – Entomologia Generalis 15: 25-32.

PEAKIN, G.J. 1972: Aspects of productivity in *Tetramorium caespitum* L. – Ekologia Polska 20: 55-63.

PEETERS, C. 2012: Convergent evolution of wingless reproductives across all subfamilies of ants, and sporadic loss of winged queens (Hymenoptera: Formicidae). – Myrmecological News 16: 75-91.

PEETERS, C. & ITO, F. 2001: Colony dispersal and the evolution of queen morphology in social Hymenoptera. – Annual Review of Entomology 46: 601-630.

PEETERS, C., KELLER, R.A. & JOHNSON, R.A. 2012: Selection against aerial dispersal in ants: two non-flying queen phenotypes in *Pogonomyrmex laticeps*. – Public Library of Science One 7: e47727.

PORTER, S.D. 1992: Frequency and distribution of polygyne fire ants (Hymenoptera: Formicidae) in Florida. – The Florida Entomologist 75: 248-257.

R CORE TEAM 2012: R: a language and environment for statistical computing. – R foundation for statistical computing, Vienna, Austria. – <http://www.R-project.org/>, retrieved on 9 July 2013.

RAYNER, J.M.V. 1988: Form and function in avian flight. – Current Ornithology 5: 1-66.

RÜPPELL, O. & HEINZE, J. 1999: Alternative reproductive tactics in females: the case of size polymorphism in winged ant queens. – Insectes Sociaux 46: 6-17.

RÜPPELL, O., HEINZE, J. & HÖLLDOBLER, B. 1998: Size-dimorphism in the queens of the North American ant *Leptothorax rugatulus* (EMERY). – Insectes Sociaux 45: 67-77.

SCHNEIDER, C.A., RASBAND, W.S. & ELICEIRI, K.W. 2012: NIH Image to ImageJ: 25 years of image analysis. – Nature Methods 9: 671-675.

SOKAL, R.R. & ROHLF, F.J. 1995: Biometry: the principles and practice of statistics in biological research. 3rd edition – W.H. Freeman and Company, New York, NY, 880 pp.

STEINER, F.M., SCHLICK-STEINER, B.C., KONRAD, H., MODER, K., CHRISTIAN, E., SEIFERT, B., CROZIER, R.H., STAUFFER, C. & BUSCHINGER, A. 2006: No sympatric speciation here: multiple data sources show that the ant *Myrmica microrubra* is not a separate species but an alternate reproductive morph of *Myrmica rubra*. – Journal of Evolutionary Biology 19: 777-787.

SUNDSTRÖM, L. 1995: Dispersal polymorphism and physiological condition of males and females in the ant, *Formica truncorum*. – Behavioral Ecology 6: 132-139.

TINAUT, A. & HEINZE, J. 1992: Wing reduction in ant queens from arid habitats. – Naturwissenschaften 79: 84-85.

TOOM, P.M., JOHNSON, C.P. & CUPP, E.W. 1976: Utilization of body reserves during preoviposition activity by *Solenopsis invicta*. – Annals of the Entomological Society of America 69: 145-148.

TSCHINKEL, W.R. 1988: Social control of egg-laying rate in queens of the fire ant, *Solenopsis invicta*. – Physiological Entomology 13: 327-350.

TSCHINKEL, W.R. 1993: Resource allocation, brood production and cannibalism during colony founding in the fire ant, *Solenopsis invicta*. – Behavioral Ecology and Sociobiology 33: 209-223.

VAN DYCK, H. & MATTHYSEN, E. 1999: Habitat fragmentation and insect flight: a changing "design" in a changing landscape? – Trends in Ecology & Evolution 14: 172-174.

VOGEL, S. 1994: Life in moving fluids: the physical biology of flow. 2nd edition. – Princeton University Press, Princeton, NJ, 484 pp.

VOGT, J.T., APPEL, A.G. & WEST, M.S. 2000: Flight energetic and dispersal capability of the fire ant, *Solenopsis invicta* BUREN. – Journal of Insect Physiology 46: 697-707.

VOSS, S.H. & BLUM, M.S. 1987: Trophic and embryonated egg production in founding colonies of the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae). – Sociobiology 13: 271-278.

WAGNER, D. & GORDON, D.M. 1999: Colony age, neighborhood density and reproductive potential in harvester ants. – Oecologia 119: 175-182.

WAGNER, D.L. & LIEBHERR, J.K. 1992: Flightlessness in insects. – Trends in Ecology & Evolution 7: 216-220.

WHEELER, D.E. & BUCK, N.A. 1996: Depletion of reserves in ant queens during claustral colony founding. – Insectes Sociaux 43: 297-302.

WHEELER, D.E. & MARTINEZ, T. 1995: Storage proteins in ants (Hymenoptera: Formicidae). – Comparative Biochemistry and Physiology 112B: 15-19.

WIERNASZ, D.C. & COLE, B.J. 2003: Queen size mediates queen survival and colony fitness in harvester ants. – Evolution 57: 2179-2183.

WIERNASZ, D.C., YENCHARIS, J. & COLE, B.J. 1995: Size and mating success in males of the western harvester ant, *Pogonomyrmex occidentalis* (Hymenoptera: Formicidae). – Journal of Insect Behavior 8: 523-531.

WINTER, U. & BUSCHINGER, A. 1986: Genetically mediated queen polymorphism and caste determination in the slave-making ant, *Harpagoxenus sublaevis* (Hymenoptera: Formicidae). – Entomologia Generalis 11: 125-138.

YAMAUCHI, K. & OGATA, K. 1995: Social structure and reproductive systems of tramp versus endemic ants (Hymenoptera: Formicidae) of the Ryukyu Islands. – Pacific Science 49: 55-68.

ZERA, A.J. & DENNO, R.F. 1997: Physiology and ecology of dispersal polymorphism in insects. – Annual Review of Entomology 42: 207-230.