

# A life history continuum in the males of a Neotropical ant assemblage: refuting the sperm vessel hypothesis

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**Abstract** Animal lifespans range from a few days to many decades, and this life history diversity is especially pronounced in ants. Queens can live for decades. Males, in contrast, are often assumed to act as ephemeral sperm delivery vessels that die after a brief mating flight—a view developed from studies of lekking species in temperate habitats. In a tropical ant assemblage, we found that males can live days to months outside the nest, a trait hypothesized to be associated with female calling, another common mating system. We combined feeding experiments with respirometry to show that lifespan can be enhanced over 3 months by feeding outside the nest. In one focal female calling species, *Ectatomma ruidum*, feeding enhanced male

lifespan, but not sperm content. Extended lifespans outside the nest suggest stronger than expected selection on premating traits of male ants, although the ways these traits shape male mating success remain poorly understood.

**Keywords** Lifespan · Mating system · Tropical rainforest · Metabolic scaling

## Introduction

Explaining why animal lifespans range from fewer than 10 days to over ten decades is a central goal of life history theory (Finch 1990; Stearns 1992). Within species, the sexes often differ greatly in lifespan as fitness in males tends to increase with mating frequency while fitness in females increases with longer lives (Trivers 1972).

Sexual lifespan divergence is especially pronounced in ants. Queens establish colonies and often live for decades (Keller and Genoud 1997). Males, in contrast, are often considered to be ephemeral sperm delivery vessels that die after a brief mating flight (Boomsma et al. 2005), although this view stems from studies of temperate ants (Baer 2011). Recent work in tropical forests suggests geographic differences in ant mating systems (Kaspari et al. 2001a) and both short-lived and long-lived syndromes in male ants outside the nest (Shik and Kaspari 2009). These results suggest that male flight ecology, though seldom studied, represents a frontier in our understanding of the ants that dominate tropical forests.

Most ant populations exhibit one of two mating systems. In *male aggregation* (MA), winged male and female reproductives (or alates) leave nests simultaneously and females enter large male-dominated swarms (Hölldobler and Bartz 1985). In the *female calling* (FC) system, winged males

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search for winged females that advertise with pheromones, often near their natal nests (Hölldobler and Bartz 1985). While the pulsed mating flights of MA are conspicuous seasonal events in temperate regions (Dunn et al. 2007), the asynchronous flight patterns of FC are common in tropical forests (Kaspari et al. 2001b). Moreover, recent experiments suggest that FC males searching for females are selected to live longer than MA males that form ephemeral leks (Shik and Kaspari 2009).

Male lifespan, therefore, can have at least two resource-based dynamics. If male lifespans depend on resources provisioned in natal colonies, then males gradually starve after leaving the nest and larger male ants should survive longer based on the *starvation resistance hypothesis* (SRH) (Lindstedt and Boyce 1985; Kaspari and Vargo 1995). This hypothesis assumes that larger males have lower mass-specific energy demands because metabolism scales as  $\text{Mass}^b$ , where  $b=0.75$ , and that body reserves scale as  $\text{Mass}^{1.0}$  such that starvation resistance scales  $M^{1.0}/M^{0.75}$  or  $M^{0.25}$  (Peters 1983; Lindstedt and Boyce 1985). While worker ant metabolism scales as  $M^{b \approx 0.75}$  (Chown et al. 2007), we know of no such data for male ants. Alternatively, the SRH should fail if FC males feed outside the nest, uncoupling longevity from preflight provisioning and thus the body size dependence of metabolic consumption of a finite resource.

If males feed outside the nest, resources collected could be used for fitness-enhancing traits in addition to survival. One possibility is that males use harvested resources to produce sperm and can thus engage in multiple matings. While most males studied to date do not produce sperm as adults (Hung and Vinson 1975; Wheeler and Krutzsch 1992; Keller and Passera 1992; but see Heinze and Hölldobler 1993), we test for this with FC males of the common ground-nesting ant *Ectatomma ruidum* (Hölldobler and Haskins 1977) that previously lived up to 30 days outside the nest when allowed to feed (Shik and Kaspari 2009). If *E. ruidum* males generate sperm outside the nest, we predict that (1) males captured in flight will have viable testes with sperm counts equal to or greater than those still in the nest and (2) since sperm are protein rich, they will have higher sperm counts when allowed to feed on nectar with soluble amino acids.

Combined, our results challenge the paradigm of male ants as ephemeral sperm delivery vessels. Instead, species from a tropical forest assemblage exhibit a life history continuum and likely a diversity of traits enabling a free-living existence outside the nest.

## Methods

We conducted field collections from May to July 2010 on Barro Colorado Island (BCI) (9°09' N, 79°51' W) in

Panama, capturing male alates using 15-W ultraviolet black lights (BioQuip, Rancho Dominguez, CA, USA) in this seasonally wet, lowland tropical forest. All rearing and metabolic work were conducted at the Smithsonian Tropical Research Institute laboratories on BCI. Vouchers are stored in the collection of M. Kaspari. For the species list, see Table 2.

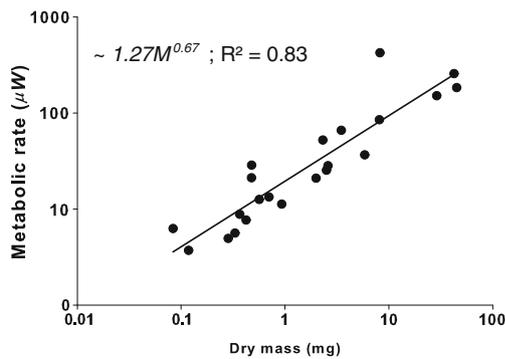
## Body size, energy demands, and starvation resistance

To assess the SRH, we first tested the assumption that larger males have lower mass-specific energy demands by measuring the metabolic rates (MR) of male ants of 10 species (selected based on availability at UV black lights). We used Sable Systems equipment to perform constant volume respirometry and measure microliters of  $\text{CO}_2$  release per hour. All MR estimates were based on an average of five hourly measurements following a 1-h acclimation period. Each data point was converted to the value at 25°C, assuming a  $Q_{10}$  of 2 (Lighton 2008). Minimal temperature corrections were needed because the mean ( $\pm 1$  SD) of 65 hourly temperature measurements was  $22.4 \pm 1.1^\circ\text{C}$ . For more information on the species analyzed, see Table S1, and for the respirometry methods, see Appendix S1. After respirometry, ants were frozen, dried at 60°C for 24 h, and weighed to the  $10^{-3}$  mg.

We supplemented these data ( $N=10$  species) with data on male ant metabolism from the literature ( $N=12$  species) (Table S1). We converted all metabolic data to microwatts, assuming joule equivalences of  $24.65 \text{ kJ L}^{-1} (\text{CO}_2)$  or  $20.7 \text{ kJ L}^{-1} (\text{O}_2)$  (as per Chown et al. 2007). Where necessary, we used a factor of 0.36 to convert wet mass to dry mass (as per Chown et al. 2007) for literature data. We used ordinary least square regression to estimate  $a$  and  $b$  in the scaling equation  $\log_{10}\text{MR} = \log_{10}a + b\log_{10}M$  (dry mass milligrams). To detect allometry ( $b \neq 1$ ), we calculated the  $F$  statistics to test the null hypothesis of isometry ( $H_0: b=1$ ).

We next tested the central prediction of the SRH—that male starvation tolerance will scale as  $M^{0.25}$ . To do this, we used data from the feeding experiment described below, regressing logarithms of average and maximum number of days that males survived when provided only  $\text{dH}_2\text{O}$  with  $\log(M)$ . We estimated the initial mass ( $M$ ) of these experimental males by collecting up to six additional randomly selected males per species, based on rarity at black lights, and freezing immediately after capture (Table 2).

We then repeated these scaling analyses after removing potential phylogenetic nonindependence from the dataset. To do this, we used the Analysis of Phylogenetics and Evolution (APE) package (Paradis et al. 2004) in R to calculate phylogenetically independent contrasts based on the topology and branch lengths of Moreau et al. (2006). For detailed methods, as well as the phylogenies used to calculate contrasts, see Supplementary Figs. S1 and S2.



**Fig. 1** Metabolic allometry for male ants across 22 species ( $\geq 17$  genera) and approximately three orders of magnitude in dry mass. Each data point represents a species mean

### Feeding behavior and longevity

To assess whether males feed outside the nest, we tested if food supplementation increases longevity in males captured in flight at UV black lights (for the species list, see Table 2). Immediately after capture, we transferred males to feeding chambers housed in a screened laboratory under ambient forest conditions. Feeding chambers were glass tubes stopped with cotton plugs soaked with either  $\text{dH}_2\text{O}$  or 10% ( $m/v$ ) sucrose solution (in  $\text{dH}_2\text{O}$ ); we replaced cotton plugs daily. We also checked survivorship daily. We sought 50 males per species, but we included in the analyses all species for which we had at least 10 individuals. For each species, we tested the effects of diet on longevity with a nonparametric Mann–Whitney test and included feeding trial results for four species examined in Shik and Kaspari (2009).

### Diet and sperm content

We next assessed the function of male feeding in *E. ruidum*, a known FC species. We first examined the potential for sperm production outside the nest by performing dissections

of *E. ruidum* males. Based on the criteria of Passera and Keller (1992), we classified testes condition as intact or degenerated in both “preflight” males from excavated nests ( $N=9$ ) and “free-living” males captured in flight ( $N=19$ ) (for details, see Fig. S3). We then tested for sperm depletion by comparing sperm number in preflight and free-living males using a hemocytometer (for details, see Supplementary Appendix S1). We confirmed the accuracy of sperm counts on a subset of males with the DAPI staining method of Stürup et al. (2011) (Appendix S1). Among free-living males examined, 21% (4 of 19) lacked sperm. We assumed that these males had already mated and removed them prior to analyses. The data were log-transformed to improve normality.

We next tested the effects of diet quality on sperm maintenance. We captured *E. ruidum* males in flight and kept individuals in tubes plugged with cotton soaked in one of four solutions: (1)  $\text{dH}_2\text{O}$ , (2) *sucrose* (10 g sugar in 100 ml  $\text{dH}_2\text{O}$ ), (3) *protein* (10 g protein: 5 g protein from whey (6.66 g whey: 75% protein; GNC) and 5 g protein from calcium caseinate (5.46 g calcium caseinate: 91.5% protein; Trueprotein) in 100 ml  $\text{dH}_2\text{O}$ ), and (4) *sucrose–protein* solution combining diets 2 and 3 in 100 ml  $\text{dH}_2\text{O}$ . These synthetic diets consist of ingredients used by Kay et al. (2011) to feed *E. ruidum* colonies in laboratory experiments. After 2, 5, and 10 days, we used hemocytometry to count the sperm of five males from each of the four diets ( $N=60$  males). We used Kruskal–Wallis tests to compare sperm counts across diets for each sampling day. Males on the protein-only diet lived at most 8 days following capture, precluding the day 10 comparison.

## Results

We found little support for the SRH and its assumption that male longevity depends only on resources provisioned by

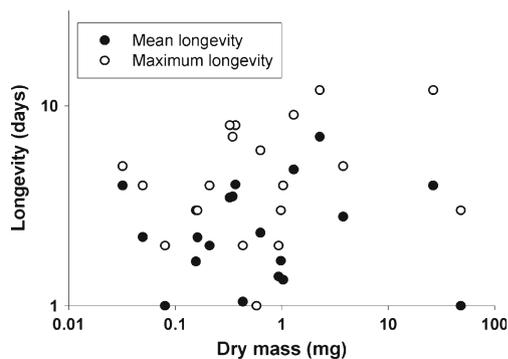
**Table 1** Results from least squares mean regression for log–log scaling of male traits with dry body mass (in milligrams)

Male trait	$N$	MS model	MS error	$F$	$R^2$	$a$	SE of $a$	$b$	SE of $b$	$\pm 95\%$ CI of $b$
MR	22	5.80	0.06	99.56	0.83	1.27	0.05	0.67**	0.07	0.14
PIC MR	14	0.01	0.00	49.79	0.79	n.a.	n.a.	0.70*	0.10	0.21
Mean longevity	20	0.00	0.00	0.01	0.00	0.35	0.06	0.00	0.07	0.16
PIC mean longevity	10	0.00	0.00	0.03	0.00	n.a.	n.a.	−0.01	0.08	0.19
Maximum longevity	20	0.09	0.09	1.15	0.06	0.65	0.07	0.09	0.08	0.17
PIC maximum longevity	10	0.00	0.00	0.84	0.09	n.a.	n.a.	0.07	0.08	0.18

PIC data are regressions of phylogenetically independent contrasts (for details, see Supplementary Figs. S1 and S2). PIC regressions lack intercepts because they intersected the origin (as per Warton et al. 2006). The overall models were not significant for the scaling of mean ( $p=0.92$ ) and maximum longevity ( $p=0.30$ ) and for PIC analyses of mean ( $p=0.87$ ) and maximum ( $p=0.38$ ) longevity

$N$  number of species in the analysis

\* $p=0.01$ , \*\* $p=0.0001$ , allometry (i.e.,  $F$  tests of deviation of slope from isometry  $b=1.0$ )



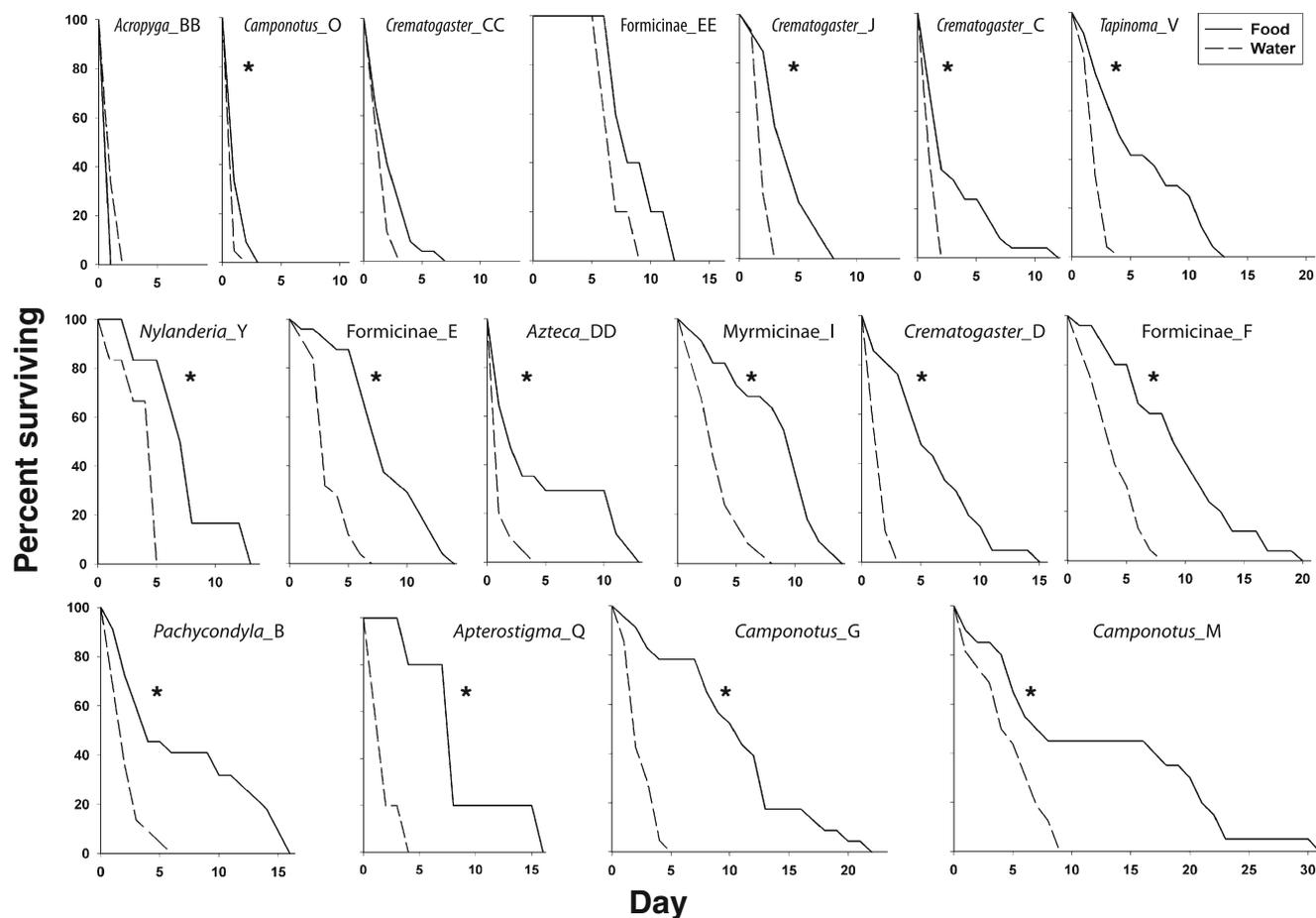
**Fig. 2** The mean and maximum longevity of male ants provided water were independent of body size. Each species represented by a *closed circle* (mean longevity) and *open circle* (maximum longevity)

the natal colony. Although MR scaled allometrically with body mass as predicted ( $\sim 1.27M^{0.67 \pm 0.14}$ ;  $R^2=0.83$ ;  $F$  test for  $b=1$ :  $F_{1,20}=24.02$ ;  $p<0.0001$ ) (Fig. 1; Table 1), longevity was unrelated to body size (Fig. 2) and thus the body size dependence of metabolic consumption. Body size explained  $<1.0\%$  of variation for mean longevity and  $6.0\%$  of

variation for maximum longevity, and neither regression was significant (Table 1). Analyses of phylogenetically independent contrasts supported both the metabolic allometry (Table 1; Fig. S1) and the lack of correlation between body size and longevity (Table 1; Fig. S2).

As further evidence against the SRH, feeding by males captured outside the nest was common in our experimental setup among the 21 species ( $>800$  males) from  $\geq 11$  genera and 6 subfamilies we studied (Fig. 3). Access to  $10\%$  ( $m/v$ ) sucrose solution extended male longevity in all but five species, with longevity for males fed sucrose ranging from 1 to 116 days (mean $\pm$ SD,  $7.6 \pm 7.3$ ) and longevity for males on  $dH_2O$  ranging from 1 to 12 days (mean $\pm$ SD,  $2.8 \pm 1.8$ ) (Table 2).

Focal studies of *E. ruidum* showed that these FC males fed to extend longevity (Table 2), but not to produce sperm. This is because free-living males always had degenerated testes (Supplementary Fig. S3). Male feeding was also unrelated to maintaining existing sperm. First, males with access to protein-rich nectar did not have higher sperm counts relative to males provided only  $dH_2O$  (Fig. 4).



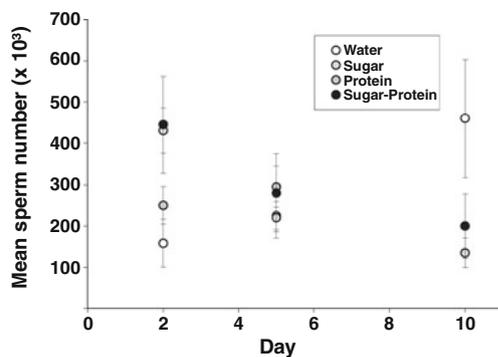
**Fig. 3** Survivorship curves comparing male longevity when maintained on  $dH_2O$  (water) or fed sucrose solution (food). Significant results of MW tests are indicated by *asterisks* and detailed in Table 1

**Table 2** Summary results from feeding experiments

Subfamily	Species/morphospecies	Capture date	Dry mass (mg)		Avg. days alive ( $\pm$ SD)		Range days alive		Mann–Whitney test		
			<i>n</i>	Avg. ( $\pm$ SD)	Control	Food	Control	Food	<i>U</i>	<i>n, n</i>	<i>p</i>
Dolichoderinae	<i>Azteca</i> sp.	5/31	6	0.58 $\pm$ 0.13	<1	<1	n.a.	n.a.	n.a.	n.a.	n.a.
	<i>Azteca</i> _DD	6/20–7/8	1	1.03	1 $\pm$ 1	5 $\pm$ 5	1–4	1–13	409.0	17, 20	<i>0.001</i>
	<i>Tapinoma</i> _V	6/3	6	0.05 $\pm$ 0.00	2 $\pm$ 1	6 $\pm$ 4	1–4	1–13	761.0	24, 24	<i>0.0001</i>
Ectatomminae	<i>Ectatomma</i> <i>rudum</i>	5/19–6/9	6	2.27 $\pm$ 0.20	7 $\pm$ 2	10 $\pm$ 7	1–12	1–33	2205.5	45, 44	<i>0.03</i>
Formicinae	<i>Acropyga</i> _BB	6/21	1	0.08	1 $\pm$ 1	1 $\pm$ 0	1–2	1–1	14	4, 3	0.20
	<i>Camponotus</i> _G	5/27–6/5	3	3.77 $\pm$ 0.80	3 $\pm$ 1	10 $\pm$ 6	1–5	1–22	240.5	23, 19	<i>0.0001</i>
	<i>Camponotus</i> _M	5/31–6/5	6	1.29 $\pm$ 0.19	5 $\pm$ 3	12 $\pm$ 9	1–9	1–31	228.0	20, 16	<i>0.02</i>
	<i>Camponotus</i> _O	6/1	6	0.43 $\pm$ 0.10	1 $\pm$ 0	1 $\pm$ 1	1–2	1–3	410.0	24, 21	<i>0.01</i>
	<i>Nylanderia</i> _Y	6/16–6/27	1	0.03	4 $\pm$ 2	8 $\pm$ 3	1–5	3–13	52.5	6, 6	<i>0.02</i>
	E	5/27	5	0.35 $\pm$ 0.02	4 $\pm$ 2	8 $\pm$ 3	1–7	1–14	844.0	24, 25	<i>0.0001</i>
	EE	6/28–7/10			7 $\pm$ 1	9 $\pm$ 3	6–9	7–14	35	5, 5	0.07
	F	5/27–6/2	6	0.37 $\pm$ 0.03	4 $\pm$ 2	9 $\pm$ 5	1–8	1–20	371.5	25, 23	<i>0.0001</i>
	Myrmicinae	<i>Apterostigma</i> _Q	6/1–6/5	1	0.21	2 $\pm$ 1	9 $\pm$ 4	1–4	4–16	39.5	5, 5
<i>Atta</i> <i>colombica</i>		5/22	6	48.04 $\pm$ 2.58	1 $\pm$ 1	2 $\pm$ 2	1–3	1–3	25.5	7, 7	0.47
<i>Crematogaster</i> _C		5/27	6	0.93 $\pm$ 0.07	1 $\pm$ 1	3 $\pm$ 3	1–2	1–12	770.0	25, 25	<i>0.003</i>
<i>Crematogaster</i> _CC		6/21	6	0.98 $\pm$ 0.10	2 $\pm$ 1	2 $\pm$ 2	1–3	1–7	717.5	25, 25	0.051
<i>Crematogaster</i> _D		5/27	6	0.16 $\pm$ 0.02	2 $\pm$ 1	6 $\pm$ 4	1–3	1–15	670.0	21, 24	<i>0.0001</i>
<i>Crematogaster</i> _J		5/29–5/31	1	0.16	2 $\pm$ 1	4 $\pm$ 2	1–3	1–8	254.5	13, 15	<i>0.001</i>
I		5/29–6/29	3	0.33 $\pm$ 0.08	3 $\pm$ 2	8 $\pm$ 4	1–8	1–14	748.5	23, 25	<i>0.0001</i>
Ponerinae	<i>Pachydondyla</i> _B	5/26–6/20	5	0.63 $\pm$ 0.04	2 $\pm$ 1	7 $\pm$ 6	1–6	1–16	597.0	21, 22	<i>0.0005</i>
Paraponerinae	<i>Paraponera</i> <i>clavata</i>	6/3–6/9	6	26.44 $\pm$ 1.80	4 $\pm$ 3	36 $\pm$ 23	1–12	3–116	62.0	10, 10	<i>0.0006</i>

All males were captured outside the nest and randomly assigned to a diet of sucrose solution (food) or dH<sub>2</sub>O (control). *Azteca* sp., *A. colombica*, *E. rudum*, and *P. clavata* capture dates from 2007. Sample size differences for mass measurements and Mann–Whitney tests reflect rarity of some species at UV black lights. Significant *p* values from Mann–Whitney tests (in italics) indicate greater longevity for males when provided food relative to control. When species or genus level identifications were not possible, letters were assigned to indicate morphospecies

Second, while dietary sperm count differences existed on day 2 ( $\chi^2=8.49$ , 3 *df*, *p*=0.04), these differences were absent by day 5 ( $\chi^2=0.81$ , 3 *df*, *p*=0.85) and day 10 ( $\chi^2=2.91$ , 2 *df*, *p*=0.23). As further evidence that sperm maintenance is independent of feeding, sperm were not depleted



**Fig. 4** Diet and sperm maintenance for males of *E. rudum*. Each data point is the mean (mean $\pm$ 1 SE) sperm count of five males dissected after being maintained on one of the four diets for 2, 5, or 10 days. Males on the protein-only diet lived at most 8 days following capture, precluding the day 10 comparison

after 10 days of starvation on dH<sub>2</sub>O (Fig. 4) nor were they depleted after males left the nest. Sperm counts of 270,000 $\pm$ 144,106 (range, 75,000 to 600,000) for free-living males did not differ from preflight counts of 369,444 $\pm$ 190,303 (range, 150,000 to 725,000) ( $t_{22,0.05}=-1.41$ ; *p*=0.17). Combined, these results suggest that the primary benefit of feeding is to extend longevity outside the nest.

## Discussion

In this, the broadest comparative study of male ant flight ecology to date, we find substantial life history variation in males from a diverse tropical forest. Moreover, males metabolized and consumed energy at rates suggesting that many species fuel a free-living existence by foraging outside the nest. However, feeding by *E. rudum* males promoted one component of fitness—lifespan, but not another—sperm production. Combined, these results suggest that a continuum of male longevity outside the nest is likely associated with a diversity of traits enabling free-living existence.

We did not find support for the SRH, which predicts that male longevity increases with body mass ( $M^{0.25}$ ). Instead, males of most of the species in our study lived longer when provided sucrose solution in our feeding experiments. However, while feeding by itself does not equate to FC, three pieces of evidence support a hypothesis that such feeding allows FC males to survive outside the nest while searching for signaling queens. First, the five nonfeeding species used male aggregation, mating in conspicuous male-dominated swarms (*A. colombica*, *Azteca* sp., *Crematogaster* CC, *Camponotus* O; JJS, personal observation) and having pulsed mating flights (*Acropyga* BB; Kaspari et al. 2001a). Second, in a previous study of males with known mating systems, two FC species lived longer when allowed to feed, but two MA species invariably died within days of capture (Shik and Kaspari 2009). Third, in contrast to temperate assemblages dominated by seasonal pulses of lekking MA males (Dunn et al. 2007), asynchronous flights occurring throughout the year are common in tropical rain forests (Kaspari et al. 2001b). More fundamentally, the life history continuum observed here complicates the common view of male ants as ephemeral sperm vessels.

Focal studies of *E. ruidum* shed light on a central aspect of the FC mating system—whether long-lived males mate multiple times. On one hand, *E. ruidum* males carry approximately 200,000 sperm or 2,000 times the number needed for a queen to reach the average mature colony size of 100 workers (Breed et al. 1990). In addition, feeding trials suggest that these sperm do not deplete in the time it takes free-living males to locate signaling queens. However, there are also reasons to predict single mating in these males. First, they lack seminal vesicles divided into an ejaculatory section and a sperm reservoir (JJS, personal observation) and may thus be physiologically unable to inseminate multiple females (Baer and Boomsma 2004). Moreover, male-biased sex ratios resulting from a population of free-living males and gradually dispersing virgin queens should minimize selection for living beyond a first successful mating (Boomsma et al. 2005).

Research on male ants tends to focus on traits they lack (e.g., reduced mandibles, metapleural glands, and telomeres; Brown 1968; Boomsma et al. 2005; Jemielity et al. 2007). However, the feeding observed across distantly related ant species suggests a diversity of adaptations for life outside the nest. For instance, short-lived MA males are thought to fuel energetically costly flights (Vogt et al. 2000) by metabolizing stored carbohydrates (Peakin 1964; Jutsum and Quinlan 1978; Passera et al. 1990). However, long-lived FC males may rely on stored lipids, like other long-distance fliers (Kammer and Heinrich 1978), and require alternative metabolic pathways. Moreover, such adaptations may vary among closely related species. For instance, the genus *Camponotus* contained both ephemeral species and

others that lived weeks when fed (Table 1). While this result is consistent with variable flight patterns for this genus (Kaspari et al. 2001a), it also contrasts studies of temperate ants that predict mating systems to be conserved at the genus level (Boomsma and Ratnieks 1996; Boomsma 2009).

Our experiments simplify what must be a more complex reality of male ants outside the nest. However, ant mating systems are described for only about 100 of 14,000 (0.7%) of species (Baer 2011), with even less known (due in part to unresolved taxonomy) of the life history of male ants. In feeding experiments, we thus assumed that males forage for resources resembling plant nectar and that food-plugged vials simulate, while simplifying the many challenges confronting males outside the nest (e.g., variable temperature and humidity, predator risk, abrasion, and energy expended flying and walking). Finally, our focus on the ecology of males after leaving the natal nest means that we may have underestimated overall male longevity, especially for MA males that wait for flights synchronized at the population level (e.g., *A. colombica*; Weber 1972; Baer and Boomsma 2006). Although more work is needed to explicitly link feeding behavior in laboratory experiments to feeding behavior in the field, and these traits to mating systems, our experimental approach provides a framework to advance our understanding of ant mating systems via the behavioral ecology of male ants.

Male postmating traits are of increasing interest, with studies of how the size and chemical contents of male accessory glands and the presence of postmating plugs affect the outcomes of sperm competition (Robertson 1995; Mikheyev 2003, 2004; den Boer et al. 2010). We suggest that male longevity (which varies among coexisting species from <1 to >100 days) and its correlates, like male dispersal ability, are of equal interest to the behavioral and population ecology of ants.

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