

The effects of age and behavioral development on honey bee (*Apis mellifera*) flight performance

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SUMMARY

A critical but seldom-studied component of life history theory is how behavior and age affect whole-organism performance. To address this issue we compared the flight performance of honey bees (whose behavioral development and age can be assessed independently *via* simple manipulations of colony demographics) between distinct behavioral castes (in-hive nurse bees vs out-of-hive foragers) and across lifespan. Variable-density gases and high-speed video were used to determine the maximum hovering flight capacity and wing kinematics of age-matched nurse bees and foragers sampled from a single-cohort colony over a period of 34 days. The transition from hive work to foraging was accompanied by a 42% decrease in body mass and a proportional increase in flight capacity (defined as the minimum gas density allowing hovering flight). The lower flight capacity of hive bees was primarily due to the fact that in air they were functioning at a near-maximal wing angular velocity due to their high body masses. Foragers were lighter and when hovering in air required a much lower wing angular velocity, which they were able to increase by 32% during maximal flight performance. Flight performance of hive bees was independent of age, but in foragers the maximal wingbeat frequency and maximal average angular velocity were lowest in precocious (7–14 day old) foragers, highest in normal-aged (15–28 day old) foragers and intermediate in foragers older than 29 days. This pattern coincides with previously described age-dependent biochemical and metabolic properties of honey bee flight muscle.

Key words: flight, development, senescence, *Apis mellifera*.

INTRODUCTION

A critical issue in life history theory is how behavior and age affect the lifetime patterns of whole-organism performance (Roff, 2007; Rose et al., 2007). Studies of this issue should ideally separate the effects of age and behavior without ambiguity, focus on performance traits that are ecologically relevant, and utilize free-living animals, whose behavior and physiology may be quite different from those of laboratory-reared counterparts (Ricklefs and Wikelski, 2002). These challenges can be met by comparing the flight performance of honey bees (*Apis mellifera*, whose behavioral development and age can be assessed independently *via* simple manipulations of colony demographics) among distinct behavioral castes and across lifespan. Flight is a principal trait (along with eusociality, memory, communication and navigation) contributing to honey bee fitness and success *via* colony-level resource acquisition. Flight is unique among these traits in that its capacity is subject to a suite of physiological changes during development, yet chronic performance of this behavior entails exposure to stressors (e.g. high temperature, reactive oxygen species, mechanical wear) that may hinder these same beneficial physiological traits and cause senescence (Roberts and Elekonich, 2005).

Adult honey bees proceed through behaviorally defined life-history stages as they age, a process of behavioral development called temporal polyethism. These insects increasingly rely on flight ability during this process, which normally involves in-hive tasks such as brood care (nursing) and hive maintenance during the first 2–3 weeks of adult life followed by a transition to tasks outside the hive, predominantly foraging, which typically last for 2–3 weeks

prior to death (Dukas, 2008). Among the many physiological and biochemical changes occurring between eclosion and the onset of foraging are a 10-fold increase in cytochrome concentrations (Herold and Borei, 1963), a doubling of thoracic glycogen levels (Fewell and Harrison, 2001; Harrison, 1986), and increased citrate synthase levels and troponin T (TnT) 10A expression (Schippers et al., 2006) that combine to yield an 8-fold increase in flight metabolic rate (up to 800 W kg⁻¹) during this period (Harrison and Fewell, 2002; Roberts and Harrison, 1999).

For many metabolically expensive behaviors such as insect flight, peak capacity is transient and progressively senesces (Carey et al., 2006; Grotewiel et al., 2005; Leffelaar and Grigliatti, 1984; Miller et al., 2008), presumably due in large part to oxidative stress and the impairment of mechanisms resisting it (Amdam and Omholt, 2002; Golden et al., 2002; Martin and Grotewiel, 2006; Seehuus et al., 2006; Sun and Tower, 1999; Vieira et al., 2000; Yoon et al., 2002; Yu and Chung, 2006). In *Drosophila melanogaster*, the frequency and duration of flight bouts as well as wing kinematic performance decrease with age beginning 1–2 weeks after eclosion (Carey et al., 2006; Leffelaar and Grigliatti, 1984; Miller et al., 2008). In house flies (*Musca domestica*), flight behavior accelerates age-dependent oxidative damage including the accrual of mitochondrial peroxide, carbonylation of select mitochondrial enzymes, and mitochondrial DNA damage, while preventing flight prevents such damage and increases longevity (Agarwal and Sohal, 1994; Sohal and Buchan, 1981; Sohal and Dubey, 1994; Yan et al., 1997; Yan and Sohal, 1998; Yan and Sohal, 2000).

Oxidative stress produced by the intense aerobic demands upon honey bee foragers is likely mitigated by upregulation of flight muscle Hsp70, catalase and CuZn superoxide dismutase (Williams et al., 2008; Wolschin and Amdam, 2007). However, the diurnal upregulation of Hsp70 and catalase (along with total antioxidant capacity) in the flight muscles of foragers subsides with age (Williams et al., 2008), and honey bee mortality sharply increases following 12–14 days of foraging experience (Dukas, 2008). Thus, oxidative stress that accrues with age, especially following the transition to foraging behavior, may accelerate senescence of flight capacity in honey bees.

The present study investigates how age and behavioral development independently affect honey bee flight capacity. We hypothesized that changes in flight capacity reflect physiological and biochemical changes in flight muscle that occur during behavioral development and with age as described above. We predicted that, independent of age, bees collecting pollen and nectar (foragers) will have greater flight capacity than bees performing brood care (nurses). We also predicted that the flight capacity of foragers will initially improve with age, reach some maximum level in intermediate-aged individuals, and senesce in older individuals. To separate the effects of age and behavioral development on normal vs maximal hovering flight capacity, we created a single-cohort colony (SCC) composed only of 1–2 day old honey bees. About 10% of bees in a SCC will transition to foraging precociously (i.e. about 1 week after eclosion) while others remain normal-aged nurses. In the following 1–2 weeks more bees transition into foraging behavior at a typical age while others remain in the hive as over-aged nurses. Thus, a SCC allows for comparisons of flight performance between age-matched groups of nurses and foragers, to assess the effects of behavior independently of age, and within behavioral castes, to assess the effects of age independently of behavior. We assayed maximal flight capacity by permitting bees to hover in a series of normoxic, variable-density gasses to determine the minimal gas density (MGD) that allowed for hovering flight (Roberts et al., 2004). A high-speed (4348 frames s⁻¹) digital video camera was used to record hovering sequences, from which we derived the following kinematics: wingbeat frequency (n), wing stroke amplitude (Φ) and wing angular velocity (ω). We found that honey bee flight capacity is limited and age independent in nurses but greatly improves at the transition to foraging behaviors. Moreover, flight capacity further improves with age if the transition to foraging is premature, and then senesces in very old foragers.

MATERIALS AND METHODS

SCC: sampling and weighing

A SCC containing 2240 European honey bee (*Apis mellifera* L.) workers was created from six frames of immature bees from three different source colonies (each derived from multiply mated queens) at the University of Nevada, Las Vegas apiary during late June, 2007. The frames were placed in an incubator (32°C, 75% relative humidity RH, 24 h dark cycle) and newly eclosed adult bees were removed every 24 h. The SCC was founded from adult bees that eclosed on 2 consecutive days. On the first of these 2 days, 1000 bees were fitted with small, unique, color- and number-coded tags (Opallitplättchen, Graze, KG, Endersbach, Germany) glued to the dorsal thorax for the purpose of individual identification. Of these bees, 400 were individually weighed immediately following tagging. The SCC was provided with an unrelated queen bee, one frame each of honey and pollen, and three empty frames for egg laying/brood development. The SCC was kept closed in an environmental chamber (30°C and 30% RH) for 5 days post-eclosion

to allow the queen to lay eggs and for maturation of the workers before being moved to the outdoor apiary to permit normal colony activity. Only tagged nurses and foragers were collected for assessment of flight capacity.

Maximal flight capacity and wing kinematics

Forager and nurse bees were assessed for maximal flight capacity. Foragers generally exit the hive at a relatively high velocity (relative to bees performing guarding behavior, or in-hive bees performing orientation or defecation flights) and in a straight-line trajectory towards the perimeter of the apiary. We intercepted individual outgoing foragers ($N=57$, ranging in age from 8 to 40 days) as they flew into a 1 quart (~1 l), clear plastic bag held approximately 30 cm from the entrance of the hive. Nurses ($N=40$, ranging in age from 8 to 27 days old) were collected from the comb using light forceps after they performed the caste-specific behavior of repeatedly sticking their heads into cells that contained larvae. We were unable to collect nurses older than 27 days from the original cohort of tagged bees because these individuals were gradually replaced by younger bees from the brood laid by the resident queen. Bees were transported to an environmental chamber maintained at a temperature of 30°C where maximal flight capacity was determined. Bees were weighed to the nearest 0.0001 g following assessment of maximal flight capacity.

The methods used to assess individual flight capacity were similar to those described previously (Roberts et al., 2004). Forager and nurse bees were immediately transferred to a flight chamber which consisted of a 5 l Erlenmeyer flask fitted with an inlet port at the base for gas perfusion and a lucite cover to prevent the bees from escaping. Bees were exposed to variable density, normoxic gas mixtures which consisted of oxygen and nitrogen and/or helium, and ranged from normodense air (21% O₂, 79% N₂; 1.21 kg m⁻³) to hypodense heliox (21% O₂, 79% He; 0.41 kg m⁻³) in 0.16 kg m⁻³ increments. The gasses were mixed using calibrated bi-metal thermo-actuated valves (low flow: Tylan FC-260; San Diego, CA, USA) and solenoid-actuated valves (high flow: Tylan FC-2910), and mixtures and flow rates were metered by an electronic flow controller (Sable Systems MFC-4; Las Vegas, NV, USA). When assessing maximal flight capacity and filming hovering flight, total gas flow rate was maintained at 1 l min⁻¹. Each trial began with air and the five hypodense gas mixtures were then administered in random order. In between gas mixtures, the flight chamber was flushed with the new gas mixture at a flow rate of 25 l min⁻¹ for 1 min to ensure complete washout. Bees were flown in each gas mixture until: (1) sustained hovering flight was observed and recorded; (2) hovering flight was attempted but failed (typically distinguished by the bee skimming across the floor of the chamber, unable to generate enough lift to hover); or (3) 3 min had elapsed, in which case the inactive bee was excluded from analysis. Bees that landed on the floor or sides of the chamber were persuaded to fly by agitating them with a small magnetic stir-bar, directed by a magnetic wand outside the chamber. Maximal flight capacity was determined as MGD, the minimal gas density that allowed hovering flight.

Honey bees hover in air and heliox using a horizontal stroke plane (Altshuler et al., 2005; Ellington, 1984); therefore, hovering flight kinematics were determined from the wing trajectories in the horizontal plane recorded by a single, high-speed (4348 frames s⁻¹) digital video camera (Vision Research, Phantom v5.1; Wayne, NJ, USA). The camera was oriented directly above the flask and focused such that the focal plane was at the center of the flask. Hence, hovering bees in focus and viewed directly through the mouth of

the flask were away from the narrow circumference(s) near the top of the flask and centered in the chamber at least five wing lengths (i.e. 50 mm) away from the chamber floor and walls. The central positioning within the chamber minimized the possibility of kinematic variation due to the boundary effect – when vortices become ‘trapped’ between the flyer and nearby surfaces (Rayner and Thomas, 1991). Ascending, descending or maneuvering flight was ignored. The digital video sequences were analyzed using customized software (Matlab, The Mathworks; Natick, MA USA) to determine the following kinematic variables for individual bees during hovering in air (referenced by subscript ‘norm’ in figures and following text) and hovering in the MGD (referenced by subscript ‘max’ in figures and following text): n (in Hz) was calculated from the duration to complete 10 successive wingbeats; Φ (in deg.) was calculated as the average of the downstroke and upstroke angular displacement for each of the 10 wingbeats; and $\bar{\omega}$ (in rad s^{-1}), the average wing angular velocity, was calculated from the duration to complete the total angular displacement of one downstroke and one upstroke for each of the 10 wingbeats.

Statistical analysis

Analysis of variance (ANOVA) was used to evaluate how body mass (M_b) differed between foragers, nurses and 1 day old bees (eclosion mass). Multivariate analysis of covariance (MANCOVA; $\alpha=0.05$) was used to determine the effect of behavioral caste, with M_b and age as covariates, on flight performance and kinematic variables. Our *post hoc* analyses consisted of evaluating specific relationships using linear or polynomial regression. Model I (least squares) linear regression was used to analyze relationships that included age or maximal flight capacity (MGD). Other relationships where both continuous variables were subject to measurement error were analyzed with model II linear (reduced major axis) regression. Because our *a priori* prediction was that flight capacity and kinematics in foragers would improve and then decline with age, we also used a 2nd order polynomial regression to test the effects of age on these variables.

RESULTS

Behavioral development and body mass

The honey bee M_b from a random sample of adult honey bees (exclusive of those used in flight assays) within 24 h of eclosion was 93.9 ± 13.3 mg (mean \pm s.d.; $N=40$). The youngest age at which bees began to forage was 8 days post-eclosion. Collection of nurses

and foragers for flight analyses began at this time and concluded at 27 days of age for nurses and at 40 days of age for foragers. M_b was significantly different between bees at eclosion, nurses and foragers (ANOVA: $F_{1,94}=376.9$; $P<0.001$), with foragers (76.0 ± 7.4 mg, $N=57$) being 42.9% lighter than nurses (133 ± 19.1 mg, $N=40$). However, age did not significantly affect M_b for either nurses (model I linear regression: $P=0.154$) or foragers (model I linear regression: $P=0.345$).

Flight performance and kinematics

There was a significant effect of behavioral caste, mass and age on flight performance and kinematics (MANCOVA: $P<0.001$, $P<0.001$, $P=0.006$, respectively; see Table 1). Behavioral caste had a significant effect on MGD (MANCOVA: $P<0.001$), with foragers being able to fly in gas densities 34% lower than nurses could, after correcting for variation in mass and age (Table 1). Approximately 20% of foragers could hover in pure heliox, while the same fraction of nurses was capable of hovering only in normal air or could not fly at all. Age had a significant effect on MGD (MANCOVA: $P<0.001$). Because our hypothesis predicted that maximal flight capacity would improve with age in young foragers and senesce in older foragers, we fitted a 2nd order polynomial curve to the MGD vs forager age data (Fig. 1); this polynomial regression was significant ($R^2=0.26$, $P<0.001$).

M_b also had a significant effect on MGD (MANCOVA: $P=0.005$). Because M_b varied greatly between the two behavioral castes, we further evaluated the relationship between M_b and MGD using linear regression (Fig. 2). MGD was independent of M_b in foragers (model II regression: $\text{MGD}_{\text{forager}}=0.619-0.001M_b$, $R^2=0.002$, $P=0.772$), but significantly increased with M_b in nurses (model II regression: $\text{MGD}_{\text{nurse}}=0.613+0.003M_b$, $R^2=0.177$, $P=0.006$). This effect was subtle, with variation in M_b explaining just 18% of variation in MGD in nurses. However, each bee in our experiment is an independent observation, and when behavioral castes were pooled, MGD significantly increased with M_b (i.e. lighter bees – primarily foragers – were better able to fly in hypodense gases), with variation in M_b explaining 66% of the variation in MGD for all bees combined (model II regression: $\text{MGD}_{\text{total}}=0.061+0.007M_b$, $R^2=0.660$, $P<0.001$).

For bees hovering in air, n_{norm} tended to decrease across M_b , but this trend was not significant (MANCOVA: $P=0.070$). However, M_b significantly affected Φ_{norm} and $\bar{\omega}_{\text{norm}}$ (MANCOVA: $P<0.001$, $P=0.014$, respectively). During hovering in air, Φ_{norm} significantly

Table 1. Multivariate analysis of covariance (MANCOVA) for the effects of caste, mass and age on flight performance

	Parameter estimates ^a (means \pm s.d.)		Caste ^b		Mass ^c		Age ^d	
	Nurse	Forager	$F_{1,93}$	P	$F_{1,93}$	P	$F_{1,93}$	P
n_{norm} (Hz)	233.7 \pm 3.7	229.1 \pm 2.7	0.63	0.429	3.36	0.070	1.37	0.244
Φ_{norm} (deg.)	121.1 \pm 2.9	108.7 \pm 2.1	7.21	0.009	15.3	<0.001	0.45	0.503
$\bar{\omega}_{\text{norm}}$ (rad s^{-1})	985.1 \pm 24.4	866.5 \pm 18.1	9.28	0.003	6.33	0.014	0.01	0.925
n_{max} (Hz)	220.6 \pm 4.3	219.9 \pm 3.2	0.01	0.923	0.12	0.733	3.07	0.083
Φ_{max} (deg.)	139.0 \pm 2.8	143.6 \pm 2.1	1.03	0.314	0.98	0.326	5.86	0.017
$\bar{\omega}_{\text{max}}$ (rad s^{-1})	1065.1 \pm 22.6	1102.6 \pm 16.8	1.08	0.302	0.59	0.446	15.83	<0.001
MGD (kg m^{-3})	0.99 \pm 0.04	0.65 \pm 0.03	19.23	<0.001	8.40	0.005	18.5	<0.001

^aLeast squares means evaluated at mass=100.3 mg, and age=19.9 days.

^bMANCOVA: Pillai's trace, $F_{7,87}=5.04$; $P<0.001$.

^cMANCOVA: Pillai's trace, $F_{7,87}=5.18$; $P<0.001$.

^dMANCOVA: Pillai's trace, $F_{7,87}=3.09$; $P=0.006$.

Significant values are in bold.

MGD, minimal gas density (maximal flight capacity); n , wingbeat frequency; $\bar{\omega}$, wing angular velocity; Φ , wing stroke amplitude; norm, flight in air; max, maximal flight in the MGD.

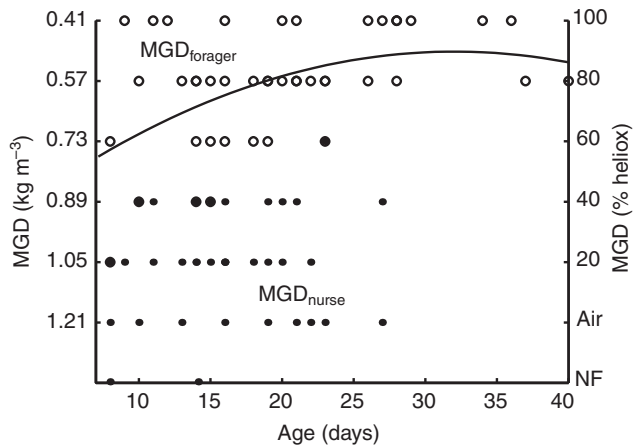


Fig. 1. Maximal flight capacity (minimal gas density, MGD) vs age for foragers (MGD_{forager} ; open symbols) and nurses (MGD_{nurse} ; filled symbols). Large filled circles indicate overlapping forager and nurse data. Values of MGD (kg m^{-3}) are inverted to reflect the increasing aerodynamic demand of flying in gas mixtures of lower density. Bees that were unable to fly in air (no flight, NF; right-hand y-axis) were plotted for descriptive purposes and were not included in the calculated MANCOVA or regressions. Second order polynomial regression for foragers: $MGD = 0.954 + 0.029\text{age} - 0.0005\text{age}^2$, $R^2 = 0.26$, $P < 0.001$ (solid line).

increased with M_b (model II regression: $P < 0.001$), with variation in M_b explaining 67% of the variation in Φ_{norm} (Fig. 3). The heaviest bees had Φ_{norm} values approximately 45% higher than the lightest bees. Likewise, $\bar{\omega}_{\text{norm}}$ significantly increased with M_b during hovering in air (model II regression: $P < 0.001$), with variation in the latter explaining 58% of the variation in the former. During hovering in the MGD, M_b did not affect n_{max} , Φ_{max} or $\bar{\omega}_{\text{max}}$ (MANCOVA: $P = 0.733$, $P = 0.326$, $P = 0.446$, respectively). Behavioral caste had a significant effect on MGD, Φ_{norm} and $\bar{\omega}_{\text{norm}}$ (MANCOVA: $P < 0.001$, $P = 0.009$, $P = 0.003$, respectively; Fig. 3). The effects of behavioral caste are similar to those of M_b (Table 1), in large part due to the significant difference in M_b between the nursing and foraging castes.

Age did not affect n_{norm} , Φ_{norm} and $\bar{\omega}_{\text{norm}}$ during hovering in air (Table 1). Although age did not affect n_{max} during hovering in the MGD, age had a significant effect on Φ_{max} and $\bar{\omega}_{\text{max}}$ (MANCOVA: $P = 0.017$, $P < 0.001$, respectively). However, the MANCOVA is a linear model and thus cannot reveal the predicted parabolic relationships between kinematic capacity and age. To test whether maximal kinematic capacities peaked in middle-aged foragers, we fitted a 2nd order polynomial curve to the forager data. The polynomial regression for n_{max} vs age was significant ($R^2 = 0.24$, $P < 0.001$) for foragers hovering in the MGD (Fig. 4A). For Φ_{max} vs age, the 2nd order polynomial regression curve fitted for foragers hovering in MGD was significant but explained only a small percentage of the variation in Φ_{max} across age ($R^2 = 0.11$, $P = 0.040$; Fig. 4B). The 2nd order polynomial curve fitted to $\bar{\omega}_{\text{max}}$ vs age was significant ($R^2 = 0.34$, $P < 0.001$) for foragers hovering in MGD (Fig. 4C). Hence, n_{max} and $\bar{\omega}_{\text{max}}$ increased with age in precocious foragers, reached a plateau in middle-aged foragers, and senesced to a small degree in older foragers. In foragers, n_{max} was less than n_{norm} , while Φ_{max} and $\bar{\omega}_{\text{max}}$ were greater than Φ_{norm} and $\bar{\omega}_{\text{norm}}$, respectively (paired t -test: $P < 0.001$ in each comparison). There were no significant regressions of n , Φ or $\bar{\omega}$ across age for nurses hovering in air or MGD. In nurses, n_{max} was slightly, but significantly, less than n_{norm} , while Φ_{max} and $\bar{\omega}_{\text{max}}$ were significantly greater than Φ_{norm} and $\bar{\omega}_{\text{norm}}$ (Fig. 5; paired t -test: $P < 0.001$ in each comparison). In

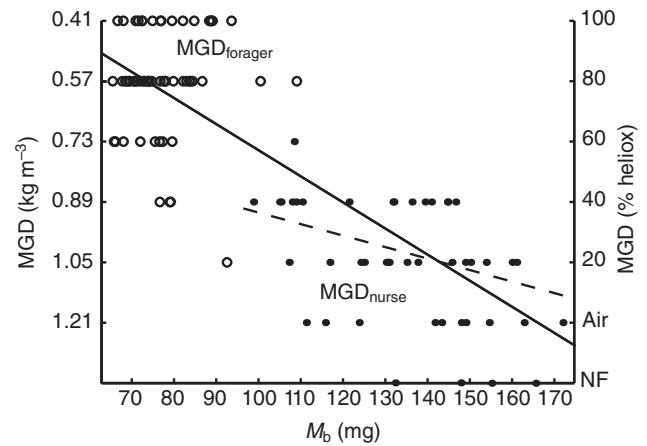


Fig. 2. Maximal flight capacity (minimal gas density, MGD) vs body mass (M_b) for foragers (MGD_{forager} ; open symbols) and nurses (MGD_{nurse} ; filled symbols). Values of MGD (kg m^{-3}) are inverted to reflect the increasing aerodynamic demand of flying in gas mixtures of lower density. Bees that were unable to fly in air (no flight, NF; right-hand y-axis) were plotted for descriptive purposes and were not included in the calculated MANCOVA or regressions. Model II regression: $MGD_{\text{forager}} = 0.619 - 0.001M_b$, $R^2 < 0.01$, $P = 0.772$; $MGD_{\text{nurse}} = 0.613 + 0.003M_b$, $R^2 = 0.18$, $P = 0.006$ (broken line). $MGD_{\text{total}} = 0.061 + 0.007M_b$, $R^2 = 0.66$, $P < 0.001$ (solid line).

order to better understand how kinematic performance might affect the caste-specific flight performance, we performed an ANCOVA to investigate the effects of caste on MGD with $\bar{\omega}_{\text{max}}$ as a covariate (Fig. 6). There was a significant interaction between caste and $\bar{\omega}_{\text{max}}$ on MGD (ANCOVA: $F_{1,93} = 5.38$, $P = 0.023$). In foragers, MGD significantly increased with $\bar{\omega}_{\text{max}}$, with variation in $\bar{\omega}_{\text{max}}$ explaining 62% of the variation in MGD. However, there was no relationship between $\bar{\omega}_{\text{max}}$ and MGD in nurses.

DISCUSSION

Using SCCs and variable-density gas mixtures, we were able to show that both age and behavioral development affect the flight performance of honey bees. To our knowledge this is the first study to experimentally segregate these factors and test their effects on the locomotor capacity of a free-living organism over a lifetime. The ability to fly in hypodense atmospheres greatly improves at the transition from nursing to foraging behaviors, and this improvement is facilitated predominantly by a large decrease in body mass that accompanies this transition. Although precocious (8–14 day old) foragers had greater flight capacity than age-matched nurses, flight capacity generally improved with age in young (15–21 days old) and typical-aged (22–28 day old) foragers. Peak kinematic performance was lowest in precocious (7–14 day old) foragers, highest in normal-aged (15–28 day old) foragers and intermediate in foragers older than 29 days. Kinematic performance and flight ability strongly increased following the transition to foraging (although this improvement was not complete if the behavioral transition occurred too early), and also showed modest, but perhaps ecologically important, signs of senescence in the oldest foragers in the study.

Body mass and flight performance

The primary basis for improved flight ability in foragers was the large (~43%) decrease in M_b that occurred prior to the transition to foraging behavior, regardless of age. The reduction in M_b prior to the behavioral transition is restricted to tissues of the abdomen and

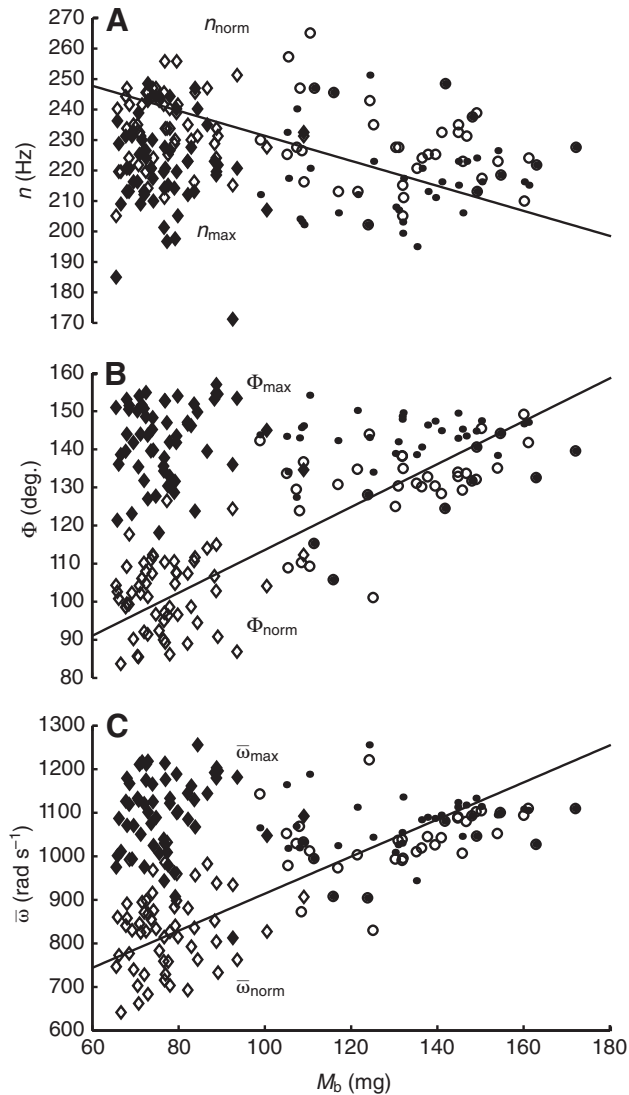


Fig. 3. Wingbeat frequency (n ; A), wing stroke amplitude (Φ ; B) and average wing angular velocity ($\bar{\omega}$; C) vs body mass (M_b) for foragers (diamonds) and nurses (circles) during flight in air (norm; open symbols) and maximal flight in the MGD (max; filled symbols). Model II regression for n : $n_{\text{norm}}=272.18-0.411M_b$, $R^2=0.07$, $P=0.008$ (solid line); $n_{\text{max}}=267.71-0.474M_b$, $R^2=0.01$, $P=0.401$. Model II regression for Φ : $\Phi_{\text{norm}}=56.85+0.568M_b$, $R^2=0.67$, $P<0.001$ (solid line); $\Phi_{\text{max}}=173.54-0.318M_b$, $R^2<0.01$, $P=0.823$. Model II regression for $\bar{\omega}$: $\bar{\omega}_{\text{norm}}=488.49+4.256M_b$, $R^2=0.58$, $P<0.001$ (solid line); $\bar{\omega}_{\text{max}}=1356.80-2.689M_b$, $R^2=0.01$, $P=0.347$.

is primarily due to gut emptying; hence, thoracic mass remains constant (but relative thorax mass increases) across the behavioral transition (Harrison, 1986). The strong effect of M_b on flight capacity was not apparent by comparing the two variables within each behavioral caste, as flight capacity was unaffected by M_b in foragers and only weakly correlated with M_b in nurses. However, when the two behavioral castes were pooled, yielding a much broader range of independent observations of mass and flight capacity in honey bees as a general group, a strong inverse relationship between M_b and MGD was revealed (Fig. 2).

Nurse bees had a very limited reserve capacity for kinematic and aerodynamic performance due predominantly to their heavy bodies but also to their immature flight muscles. While hovering in air, the

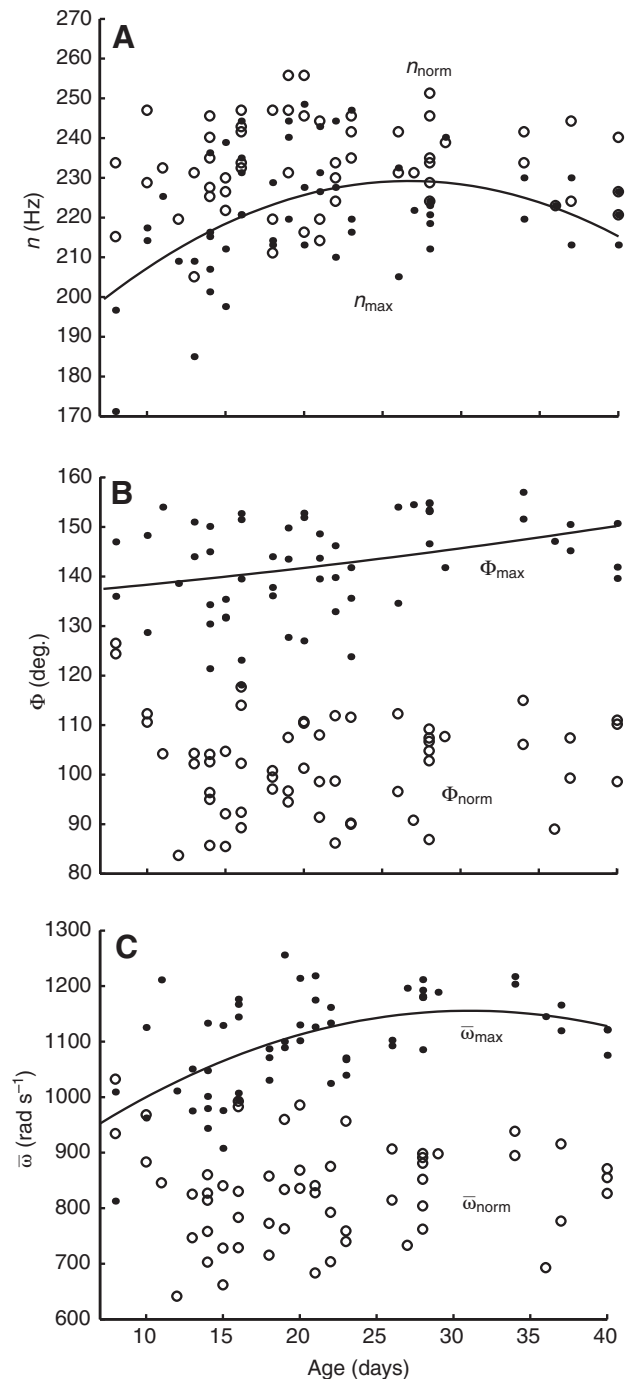


Fig. 4. Wingbeat frequency (n ; A), wing stroke amplitude (Φ ; B) and average wing angular velocity ($\bar{\omega}$; C) vs age for foragers. Second order polynomial regression for n_{max} : $n_{\text{max}}=173.27+4.19\text{age}-0.079\text{age}^2$, $R^2=0.24$, $P<0.001$. Second order polynomial regression for Φ_{max} : $\Phi_{\text{max}}=135.57+0.252\text{age}-0.003\text{age}^2$, $R^2=0.11$, $P<0.001$. Second order polynomial regression for $\bar{\omega}_{\text{max}}$: $\bar{\omega}_{\text{max}}=813.50+22.14\text{age}-0.355\text{age}^2$, $R^2=0.34$, $P<0.001$ (solid line).

Φ_{norm} and $\bar{\omega}_{\text{norm}}$ of heavier, younger bees (nurses) were at or just below maximal attainable levels. Moreover, nurses were unable to sustain normal n when challenged to hover in hypodense gases – to the extent that $\bar{\omega}_{\text{max}}$ exceeded $\bar{\omega}_{\text{norm}}$ by only 8% (after adjusting for the effects of age and mass). Precocious foragers and very old foragers were similarly unable to maintain n when challenged with

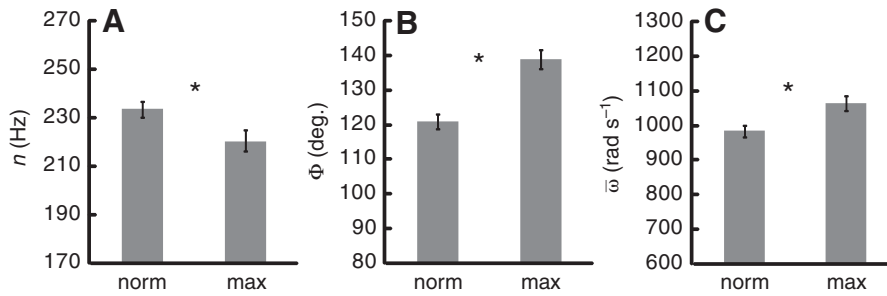


Fig. 5. Wingbeat frequency (n ; A), wing stroke amplitude (Φ ; B) and average wing angular velocity ($\bar{\omega}$; C) for nurses during hovering in air (norm) and hovering in the MGD (max). Asterisks indicate significant differences between normal and maximal hovering for n , Φ and $\bar{\omega}$ (paired t -test: $P < 0.001$ in each case).

hypodense gases, but their ability to strongly increase Φ still offered greatly elevated $\bar{\omega}$ during maximal hovering performance. When challenged with hypodense gases, only middle-aged foragers were able to increase Φ and maintain n . Hummingbirds (Althuler and Dudley, 2003; Chai et al., 1997), euglossine bees (Dudley, 1995) and carpenter bees (Roberts et al., 2004) similarly increase Φ and maintain (or even slightly increase) n during maximal hovering flight. Although there is no information on the age dependence of flight performance in these taxa, it seems plausible that kinematic performance might be similarly affected in very young or old individuals.

Across closely related hovering insects, n decreases with M_b during hovering flight (Dillon and Dudley, 2004; Dudley, 2000), but this negative relationship does not always hold true for the few available datasets allowing intraspecific comparisons of n and M_b . In honey bees, there is a slight negative relationship between n_{norm} and M_b , although this is unlikely to be due to resonance issues and an increase in the induced power required to move a larger wing (factors typically associated with the negative relationship between n and M_b across similar species) because neither wing size nor thorax dimensions differ between foragers and nurses (J.T.V., unpublished observation). Instead, the heaviest honey bees (nurses) require elevated Φ just to fly in air, but their immature flight muscles do not allow them to reach n values attainable by many (particularly middle-aged) foragers, which are much lighter than nurses. For carpenter bees (*Xylocopa varipuncta*) hovering in air, heavier individuals have higher Φ (as do honey bees; Fig. 3B) and n due to disproportionately heavier abdomens and high wing loading (Roberts et al., 2004), although peak kinematic performance and M_b are independent of each other in both of these species. This is not the case during flight in heliox and maximal load lifting across several species of euglossine bees whose M_b span over an order of magnitude, in which case Φ_{max} is highly conserved near 140 deg., but n_{max} decreases with M_b (Dudley, 1995; Dillon and Dudley, 2004).

Variation in M_b was smallest in foragers, and M_b had no effect on MGD in this group. This is not so for *X. varipuncta*, in which body mass varies by 3-fold, with lighter individuals capable of hovering in lower gas densities than heavier individuals due to lower wing loading, relatively larger flight muscles and smaller abdomens (Roberts et al., 2004). The ability of honey bee foragers to fly in hypodense gases was positively correlated with $\bar{\omega}_{\text{max}}$ (Fig. 6). In several species of *Drosophila*, aerodynamic forces scale to the square of wing translational velocity (Lehmann and Dickinson, 1998), which is determined by $\bar{\omega}$, and hence it is not surprising that the honey bees capable of generating the highest values of $\bar{\omega}_{\text{max}}$ were also the ones capable of hovering in the lowest gas densities. To our knowledge this is the only study to date linking individual variation in kinematic capacity (in this case largely due to age plus random effects) to peak flight performance.

The development and senescence of flight performance

The improvement of flight muscle performance at the transition to foraging and during foraging (if the transition is premature) is likely to be due to a suite of biochemical and structural changes in the flight muscle that occur during honey bee maturation and behavioral development. For example, young honey bees (~3 days old) that have acquired the ability to fly express an isoform of TnT similar to the 46 kDa TnT localized only to the mature flight muscle of adult *Drosophila* (Domingo et al., 1998). This TnT isoform is absent in juvenile stages in *Drosophila* as well as in 1–2 day old bees that are unable to fly, suggesting that the muscle function necessary for flight is dependent upon the expression of specific TnT isoforms. Furthermore, honey bee foragers express more TnT 10A (>2-fold increase) in their flight muscles than younger hive bees (Schippers et al., 2006). The effects of the differential TnT isoform expression on honey bee flight are unknown, but in the dragonfly *Libellula pulchella* the differential expression of TnT isoforms affects flight muscle calcium sensitivity and is correlated with an increase in wingbeat frequency and amplitude as the dragonflies progress from the teneral stage to sexual maturity (Fitzhugh and Marden, 1997; Fitzhugh et al., 1999; Marden et al., 2001; Marden et al., 1998; Marden et al., 1999). Elevated TnT 10A expression may contribute to the age- and behavior-dependent increase in maximal wingbeat frequency in honey bees, and attempts to determine whether the expression of TnT isoforms (and other flight-motor proteins) is similarly affected by age and behavioral development are ongoing in our laboratories.

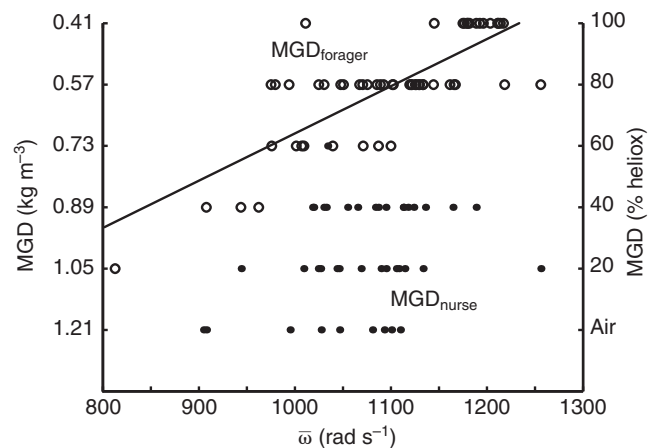


Fig. 6. Maximal flight capacity (minimal gas density: MGD) vs maximal wing angular velocity ($\bar{\omega}_{\text{max}}$) for foragers ($\text{MGD}_{\text{forager}}$; open symbols) and nurses ($\text{MGD}_{\text{nurse}}$; filled symbols). Values of MGD (kg m^{-3}) are inverted to reflect the increasing aerodynamic demand of flying in gas mixtures of lower density. Model 1 regression: $\text{MGD}_{\text{forager}} = 1.930 - 0.0012\bar{\omega}_{\text{max}}$, $F^2 = 0.62$, $P < 0.001$ (solid line); $\text{MGD}_{\text{nurse}} = 1.594 - 0.0006\bar{\omega}_{\text{max}}$, $F^2 = 0.08$, $P = 0.083$.

The reduction in maximal kinematic and flight capacity in the older foragers likely reflects senescence *via* oxidative stress within the flight muscles. The intense aerobic metabolism of forager flight muscle (over 2000 W kg⁻¹ muscle) yields high levels of reactive oxygen species, the effects of which are mitigated by the upregulation of stress and antioxidant proteins such as Hsp 70, catalase and CuZn superoxide dismutase (Schippers et al., 2006; Williams et al., 2008; Wolschin and Amdam, 2007). However, resistance to oxidative stress declines with age, as old (30–32 days) honey bee foragers express less catalase and have lower total antioxidative capacity than precocious foragers (Williams et al., 2008). Cytochrome *c* oxidase activity also decreases in aged honey bee flight muscle (Schippers, 2006), but other cellular pathologies of honey bee flight muscle senescence are unknown. In *Drosophila* and other dipterans, such pathologies include depressed actin transcription, decreased sarcomere length, enlarged/degraded mitochondria, depressed mitochondrial respiration and depressed aconitase activity (Ferguson et al., 2005; Labuhn and Brack, 1997; Miller et al., 2008; Yarian and Sohal, 2005).

The mechanical wear of wings has also been implicated as an important factor contributing to the senescence of flight performance and mortality in eusocial bees (Cartar, 1992; Dukas, 2008; Hedenstrom et al., 2001; Higginson and Barnard, 2004). These authors hypothesize that degraded wings in older bees limit flight performance with consequences for foraging ability and predator evasion. Wing wear was not a factor contributing to senescence of flight performance in our study because in our experiments we only assayed bees that possessed intact, unworn wings. However, our finding of impaired n_{\max} in very old foragers may compound the problems of worn wings. For example, bumblebees increase n in response to wing clipping (Hedenstrom et al., 2001), and such compensation may be unavailable to older honey bee foragers. We have no information about the foraging history of the bees in our study (i.e. we know the absolute age of foragers, but not how long they had been foraging), but we believe that the declines in n_{\max} , $\bar{\omega}_{\max}$ and maximal flight capacity in the older foragers probably mark the onset of senescence in the flight muscle. The pace of senescence of overall flight ability is still unknown but should be a function of both flight muscle and wing degradation.

The ecological significance of honey bee flight performance

A honey bee colony can shift worker demographics in response to a deficiency of workers in a particular caste (Huang and Robinson, 1992; Robinson et al., 1989) or worker effort in response to a shortage of pollen stores (Fewell and Winston, 1992). Such shifts might involve precocious or very old foragers, both of which have reduced maximal flight capacity, and negatively affect foraging loads and rate of foraging intake (Higginson and Barnard, 2004; Schippers et al., 2006), with potential consequences for colony-scale economy and energy flux (Schmid-Hempel et al., 1985). Likewise, colony-level intake should be higher when, all else being equal, the foraging caste is represented by middle-aged individuals. Indeed, the amount of food collected per trip increases by over 300% throughout a bee's first week of foraging behavior (Schippers et al., 2006). Finally, precocious and aged foragers may be subject to a higher predation risk due to their limited burst flight capacities (Cartar, 1992; Dukas, 2008). There are no data to confirm this linkage in honey bees, although wing damage resulting from male–male combat in the burrowing bee *Amegilla dawsoni* increases the risk of predation by birds and shortens longevity (Alcock, 1996).

Certain honey bee genotypes are predisposed to early or late initiation of foraging (Calderone and Page, 1988; Giray and

Robinson, 1994), and it is possible that the trajectory of the age-dependent development of maximal flight capacity varies genetically as well. For colonies genetically predisposed to begin foraging at an earlier age, any potential colony-level costs of precocious foraging may be mitigated by a faster rate of development and shorter periods of sub-optimal maximal flight capacity. Conversely, in colonies predisposed to a later onset of foraging, the costs of precocious foraging may be prolonged by a slower rate of development, or foraging onset may be temporally coordinated with slower development of flight capacity. Experiments addressing the temporal kinetics of foraging initiation and flight capacity among such genotypes would be valuable to test these possibilities.

Conclusion

The development of the flight capacity necessary for effective foraging in honey bees depends upon the sharp reduction in body mass at the transition from nursing to foraging behavior. Following this transition, the age-dependent development and senescence of maximal flight capacity in foragers reflects the ability, when aerodynamically challenged, to increase Φ while simultaneously maintaining n . Importantly, our experiment does not allow us to determine whether the timing of the initiation of foraging affects the onset and pace of senescence (which would require lifetime ethography of individual bees), although precocious foraging does shorten lifespan (Rueppell et al., 2007). Even so, our results suggest that variation in honey bee flight capacity across age is an important factor explaining known life-history patterns of foraging behavior and mortality rates. However, future research is needed to directly link the ontogeny of flight capacity to foraging efficacy, predation risk and mortality.

LIST OF ABBREVIATIONS

M_b	body mass
MGD	minimal gas density
n	wingbeat frequency
SCC	single-cohort colony
Φ	wing stroke amplitude
$\bar{\omega}$	wing angular velocity

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