THESIS

THE EFFECTS OF INDIVIDUAL-LEVEL DIFFERENCES IN METABOLIC RATE ON FORAGING BEHAVIOR AND LIFE HISTORY TRAITS IN HONEYBEES

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ABSTRACT

THE EFFECTS OF INDIVIDUAL-LEVEL DIFFERENCES IN METABOLIC RATE ON FORAGING BEHAVIOR AND LIFE HISTORY TRAITS IN HONEYBEES

Metabolic rate is the rate at which organisms process energy and is often considered as the fundamental driver of life history processes. The link between metabolic rate and life history is primarily mediated *via* foraging, which shapes the energy acquisition patterns of an individual. This predicts that individuals with different metabolic rates likely vary in their foraging strategies, although such links have rarely been empirically investigated in the context of optimal foraging theory - a powerful framework for understanding how animals maximize their foraging returns. Many central place foragers such as honeybees maximize their energetic efficiency rather than the rate of energetic gain, given the critical role of energetic costs on foraging decisions. We therefore tested if individuals with low or high metabolic rates differ in efficiency maximization, using genetic lines of honeybees with different metabolic rates. Our results show that low metabolic rate foragers visit more flowers during a single foraging trip and have higher energetic efficiency than high metabolic rate foragers in both low and high resource conditions. We discuss the significance of these results in the context of division of labor and the adaptive role of phenotypic diversity in metabolic rate in a social insect colony.

We then tested the rate of living hypothesis in honeybees using the same phenotypic lines of bees with low and high metabolic rate and by combining it with radio frequency identification (RFID) technology and respirometry to measure life history and metabolic rate parameters, we

specifically examined the relationships between metabolic rate and various parameters that define foraging behavior and life history. Our results show low repeatability of metabolic rate and an inconsistent effect of metabolic rate. While metabolic rate was negatively correlated with age of ontogenetic shift and lifespan in wild-type bees as predicted by the Rate of Living hypothesis, it did not show any correlation with foraging parameters. In the phenotypic lines, metabolic rate affected life history parameters in the opposite direction than what is predicted. This was accompanied by a strong effect of seasonal effect. We provide a likely explanation for these trends with a strong recommendation for integrating such environmental interactions in our understanding of the relationship between metabolic rate and life history.

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CHAPTER 1: METABOLIC RATE SHAPES DIFFERENCES IN FORAGING EFFICIENCY AMONG HONEYBEE FORAGERS

Introduction

Metabolic rate is the rate at which organisms consume, process, and expend energy and it is therefore often considered to be the fundamental driver of structure and function throughout all levels of biological organization (Brown 2004). At the level of an individual, metabolic rate shapes energy budgets by determining the rates of the various physiological and behavioral mechanisms that contribute to its energy acquisition and allocation. Metabolic rate is therefore often hypothesized to be the primary underlying driver of differences in life history and behavior at both intra- and interspecific levels (Careau and Garland 2012; Le Galliard et al. 2013) and the foundation of the Pace of Life Syndrome (POLS) which seeks to unify these phenotypic associations along a single slow-fast axis (Ricklefs and Wikelski 2002; Réale et al. 2010). However, it is important to recognize that the observed metabolic rate of an animal is also subject to the feedback effects of its behavioral and physiological states, making it imperative to get a comprehensive understanding of the complex relationship between metabolic rate and behavior.

The link between metabolic rate and life history can be directly attributed to the dynamics of foraging behavior by which an animal acquires the energetic resources that fuel its life processes, including the energy needed for foraging itself (Biro and Stamps 2010). This means that interindividual variation in metabolic rate is likely reflected in their foraging strategies with important consequences for life history (Burton et al. 2011). Optimal foraging models have been a cornerstone for understanding how foragers collect food in a manner that maximizes their

energetic returns and thereby fitness (Charnov 1976; Pyke et al. 1977), including a few that explicitly connect optimal foraging with life history (Abrams 1983). However, despite the recent interest in the role of metabolic rate and behavior on personality and pace of life (Laskowski et al. 2021), optimal foraging models have not been leveraged enough to get insights about how interindividual differences in metabolic rate, by influencing foraging strategies, could contribute to differences in behavior and life history (Houston 2010, Houston and McNamara 2014).

Social groups such as honeybee colonies, in which there is significant variation among the workers in terms of metabolic rate (Mugel and Naug 2020), offer natural "common-garden" setups to understand how interindividual differences in metabolic rate could lead to possible differences in forging strategies. In honeybees, differences in metabolic rate are known to be associated with important aspects of foraging behavior such as flight speed, lifting capacity, and other aerodynamic variables that govern foraging dynamics (Feuerbacher et al. 2003). In the context of optimal foraging, a honeybee forager, rather than maximizing its net rate of energy gain, maximizes its energetic efficiency, or the net energetic gain per unit of metabolic expenditure (Schmid-Hempel et al. 1985). The rationale for this was linked to the idea that honeybees have a fixed maximum lifetime supply of energy available for foraging (Neukirch 1982) and maximizing efficiency is likely to extend its foraging lifespan and maximize its lifetime contribution to colony resource intake. This suggests that individual level differences in physiology and energetics are likely to influence choice of foraging strategy and in turn have a significant impact on life history at the individual level (physiological senescence and lifespan) and at the colony-level (investment in maintenance and reproduction), depending on the nature of worker phenotypes (Schmid-Hempel 1987; Schmid-Hempel and Wolf 1988; Wolf et al. 1989; Wolf and Schmid-Hempel 1990;

Ydenberg and Schmid- Hempel 1994). In addition, the strategy adopted by a worker could also be context dependent with workers from small colonies maximizing gain rate and those from large colonies maximizing efficiency (Fewell et al. 1991).

Variation in metabolic rate plays a fundamental role in driving the covariance among physiological, behavioral, and life history traits in honeybees, a phenotypic covariance that has important implications for division of labor and social evolution (Mugel & Naug 2020). With this background, we tested if differences in metabolic rate are reflected in how bees maximize different foraging currencies in an effort to link interindividual differences in metabolic rate and life history to ideas in optimal foraging. We used the optimal foraging model of Schmid-Hempel et al. (1985) to predict foraging differences between honeybees of low and high metabolic rate and based on the solution of the model, we predicted that Slow bees with a low metabolic rate and Fast bees with a high metabolic rate will both optimize efficiency as a foraging currency and that Slow bees will have a higher efficiency and therefore visit more flowers on a foraging trip than Fast bees.

Methods

Experimental design

Based on the well-known variation in malate dehydrogenase (MDH-1) allotypes in honeybees, in which the Slow (S) and the Fast (F) alleles are associated with low and high metabolic rate, respectively (Harrison et al. 1996, Harrison and Fewell 2002, Feuerbacher et al. 2003), we bred and established 12 source colonies of the honeybee *Apis mellifera*, half of which produced Fast homozygous (FF) workers and the other half produced Slow homozygous (SS) workers. Fast (FF) colonies were headed by FF queens mated with F drones and Slow (SS) colonies

were headed by SS queens mated with S drones (for details, see Mugel and Naug *In press*). We then implemented a common garden design by establishing a micronucleus experimental colony with a wild-type queen and continuously populating it with introductions of newly emerged Fast (FF) and Slow (SS) bees every other week. For the introduction of each cohort, we extracted brood from the FF and SS source colonies and hatched them in an incubator set to 32 °C, paint marked about 200 (100 Fast and 100 Slow) bees based on their respective phenotype and cohort identity and introduced them into the experimental colony which consisted of about 1000 bees.

Metabolic rate measurement

We collected Fast and Slow foragers as they departed the hive on foraging trips and used carbon dioxide respirometry to measure their flight and resting metabolic rates using a FoxBox setup (Sable Systems). For the measurement of flight metabolic rate, each bee was placed in a clear 250 mL sealed glass chamber maintained at 28 °C and ambient air scrubbed of H_2O and CO_2 was run through the chamber at a constant rate of 750 mL/min for 10 min. The CO_2 concentration in the excurrent airflow was recorded every second and corrected for drift by subtracting baseline CO_2 readings taken prior. Flight was stimulated by shining a light above the chamber and lightly agitating the chamber as necessary. The behavior of the bee was monitored constantly throughout the assay and flight metabolic rate was calculated using the 60 seconds of continuous flight with the most stable (lowest variance) CO_2 production. Bees that did not fly for 60 continuous seconds were not used in the data analysis. Following the flight metabolic rate assay, each bee was harnessed in a plastic straw using a small wire, satiated with 30% sucrose and maintained in an incubator at 28 °C overnight to ensure a post-absorptive state. Each bee was then placed in a dark 50 mL chamber and its resting metabolic rate was calculated as the continuous 2-min period with

the lowest average CO_2 production. Finally, both flight and resting metabolic rates were calculated by transforming the CO_2 production (mL hr^{-1}) into a power output (W) by multiplying it by 21.4 J mL^{-1} CO_2 and dividing by 3600 J hr^{-1} (Feuerbacher et al. 2003, Mugel and Naug 2020).

Measurement of foraging parameters

Based on the experimental design of Schmid-Hempel et al. (1985), Fast and Slow foragers were trained to a foraging arena in a shaded area, 100m from the hive, with 3 artificial flowers, each with 5µl of sucrose reward (Fig SA.1). The flowers were raised off the base of the arena so that foragers were forced to fly between flowers. Once the foragers were trained, they were individually marked and let into the arena one at a time and forage on the artificial flowers which were continuously refilled after every visit, allowing the forager to visit as many flowers as it wanted to during a single foraging trip. Foragers were tested under two experimental treatments, high resource (50% w/w sucrose) and low resource (30% sucrose) conditions. Two observers, one stationed at the hive entrance/exit and the other at the foraging arena, recorded departure and arrival times of these individually marked foragers at both these locations to measure individual trip times. The behavioral details of each foraging bout within the foraging arena were video recorded to quantify the parameters for a central place foraging model that can be used to calculate the energetic gain and cost of a honeybee forager during a single foraging trip (Schmid-Hempel et al. 1985). The foraging experiments were conducted between 10am and 1pm during which the average ambient temperature varied between 26-30 °C.

From these observations and recordings, we quantified the number of flowers visited in each bout (N), the time spent at each flower or handling time (h), the time spent flying between two successive flowers (τ) and one-way travel time from the hive to the arena (τ_0). Combining

these measurements with the flight metabolic rate (a_0), the resting metabolic rate (a_h) (both measured for Slow and Fast foragers separately as described above), the linear increment of metabolic rate as a function of load weight (a) (assumed to be the same as the value used by Schmid-Hempel et al. (1985) in their study), the weight-specific energetic value of sucrose (e) and the nectar load per flower (ω), two foraging currencies, *gain rate* (*G-C/T*) and *efficiency* (*G-C/C*), were evaluated for Slow and Fast foragers, as per the Schmid-Hempel et al. (1985) model (see supplement for details), where,

the energetic gain in a single foraging trip is given by

$$G = N \cdot e \cdot \omega$$

and the energetic cost (C) in a single foraging trip is given by

Patch Cost (C_P) + Travel Cost (C_T)

where,

$$C_p = a_0 \cdot (N-1) \cdot \tau + a \cdot \frac{N \cdot (N-1)}{2} \cdot \omega \cdot \tau + a_h \cdot N \cdot h$$

$$C_T = a_0 \cdot \tau + (a_0 + a(N \cdot \omega)) \cdot \tau_0$$

and the time spent in a single foraging trip (T) is given by

$$T = 2 \cdot \tau_0 + (N-1) \cdot \tau + N \cdot h$$

Statistical analysis

A replica of the foraging model of Schmid-Hempel et al (1985) was built using the R package "shiny" (version 1.6.0, Chang et al. 2021) and simulations were run to predict the foraging efficiency and gain rate values for different number of flowers visited (N), using metabolic rate values representing high and low metabolic phenotypes and the foraging parameters measured in the original experiment. Using simulated gamma distributions for the measured parameters, a

bootstrapping procedure with 1000 iterations was used to solve the foraging model and calculate the efficiency and gain rate as a function of number of flowers visited (N) with 95 percent confidence intervals.

A linear model was used to test the effect of metabolic rate phenotype (Fast or Slow) on flight and resting metabolic rates. A general linear model was used to test the effects of phenotype and resource condition on the number of flowers visited (N), one-way travel time (τ_0), handling time (h), and the inter-flower time (τ). Pairwise comparisons using Tukey adjusted p-values were used to test for differences between the number of flowers visited by the two phenotypes. All statistical analyses were performed in R (version 4.0.5, R core team).

Results

Metabolic rate measurement

Fast foragers had both a significantly higher flight metabolic rate (One-way ANOVA $F_{1,31}$ = 4.8, p = 0.03; Fig 1.1A) and resting metabolic rate (One-way ANOVA $F_{1,77}$= 4.1, p = 0.04; Fig 1.1B) than Slow foragers.

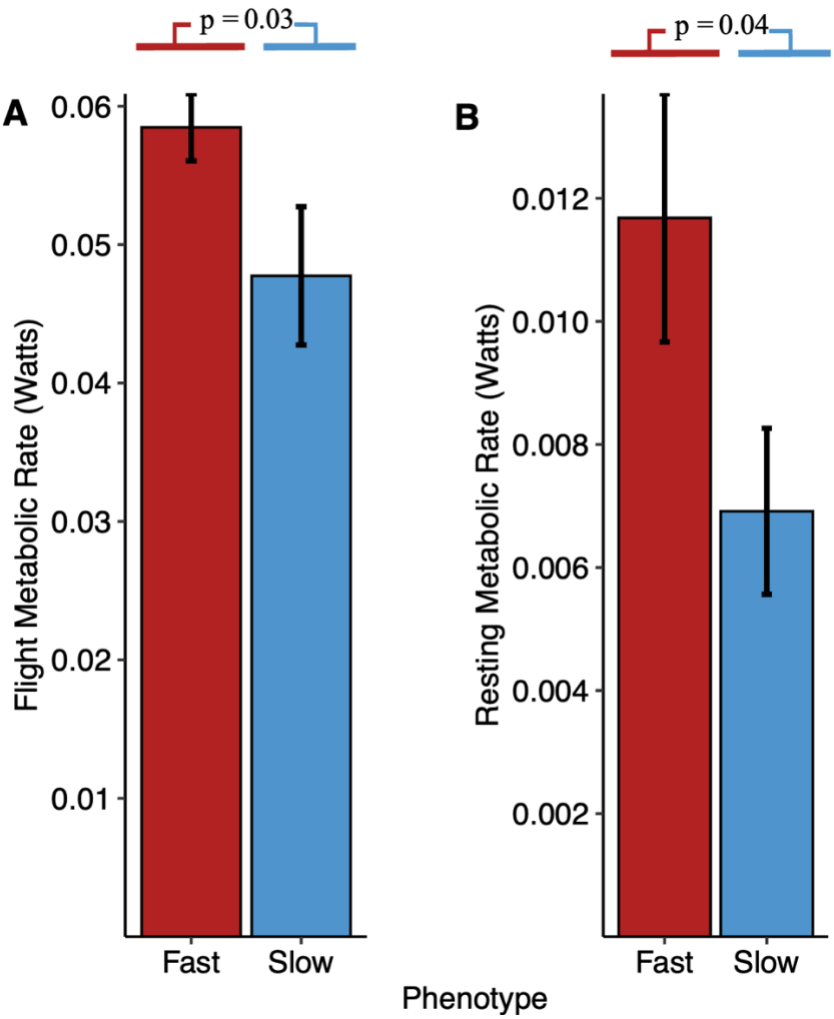


Figure 1.1. Difference between Fast and Slow foragers in (A) flight metabolic rate (Fast: n = 18; Slow: n = 14), and (B) resting metabolic rate (Fast: n = 35; Slow: n = 43), with data representing mean ± s.e.

Measurement of foraging parameters

The time spent flying from the hive to the foraging arena (one-way travel time) was recorded for 449 total foraging trips and it was significantly different between the two phenotypes but not influenced by the resource condition: 30% vs 50% sucrose (Two-way ANOVA: Phenotype: $F_{1, 446} = 10.14$, $p = 0.001$; Resource condition: $F_{1,446} = 0.001$, $p = 0.97$; Interaction = $F_{1,446} = 0.07$, $p = 0.79$; Fig 1.2A). Post-hoc comparisons for one way travel time show a significance between

Fast and Slow foragers within Low (Tukey adjusted pairwise comparisons: $t_{446} = -3.185$, $p = 0.008$) and High (Tukey adjusted pairwise comparisons: $t_{446} = -3.185$, $p = 0.008$) resource conditions.

We recorded the details of 299 total foraging trips in the foraging arena of which 185 were by Fast bees and 114 were by Slow bees. The time spent by foragers flying between consecutive flowers (inter-flower time) was not affected by either phenotype, resource condition or the interaction between the two (Phenotype: $F_{1, 296} = 0.57$, $p = 0.44$; Resource condition: $F_{1, 296} < 0.001$ $p = 0.99$; Interaction $F_{1, 296} = 0.59$, $p = 0.44$). The time spent by a forager consuming nectar at each flower (handling time) was also not influenced by phenotype, resource condition or the interaction between the two (Phenotype: $F_{1, 296} = 0.02$, $p = 0.88$; Resource condition: $F_{1, 296} = 1.66$, $p = 0.19$; Interaction = $F_{1, 296} = 0.008$, $p = 0.929$). There was a significant effect of both phenotype (Fast or Slow) and resource condition (Low or High) on the number of flowers visited by a forager during a single foraging trip (Two-way ANOVA; Phenotype: $F_{1,296} = 9.16$, $p = 0.003$, Resource condition: $F_{1, 296} = 10.77$, $p = 0.001$; Fig 1.2B), while there was no significant interaction between the two ($F_{1, 296} = 0.01$, $p = 0.91$). The post-hoc pairwise comparisons showed that of the bees foraging in Low resource conditions, Slow foragers visited more flowers than Fast foragers (Tukey adjusted pairwise comparisons; $t_{296} = -3.026$, $p = 0.0143$). Similarly, for bees foraging under High resource conditions, Slow foragers also visited more flowers than Fast foragers (Tukey adjusted pairwise comparisons; $t_{296} = -3.026$, $p = 0.0143$).

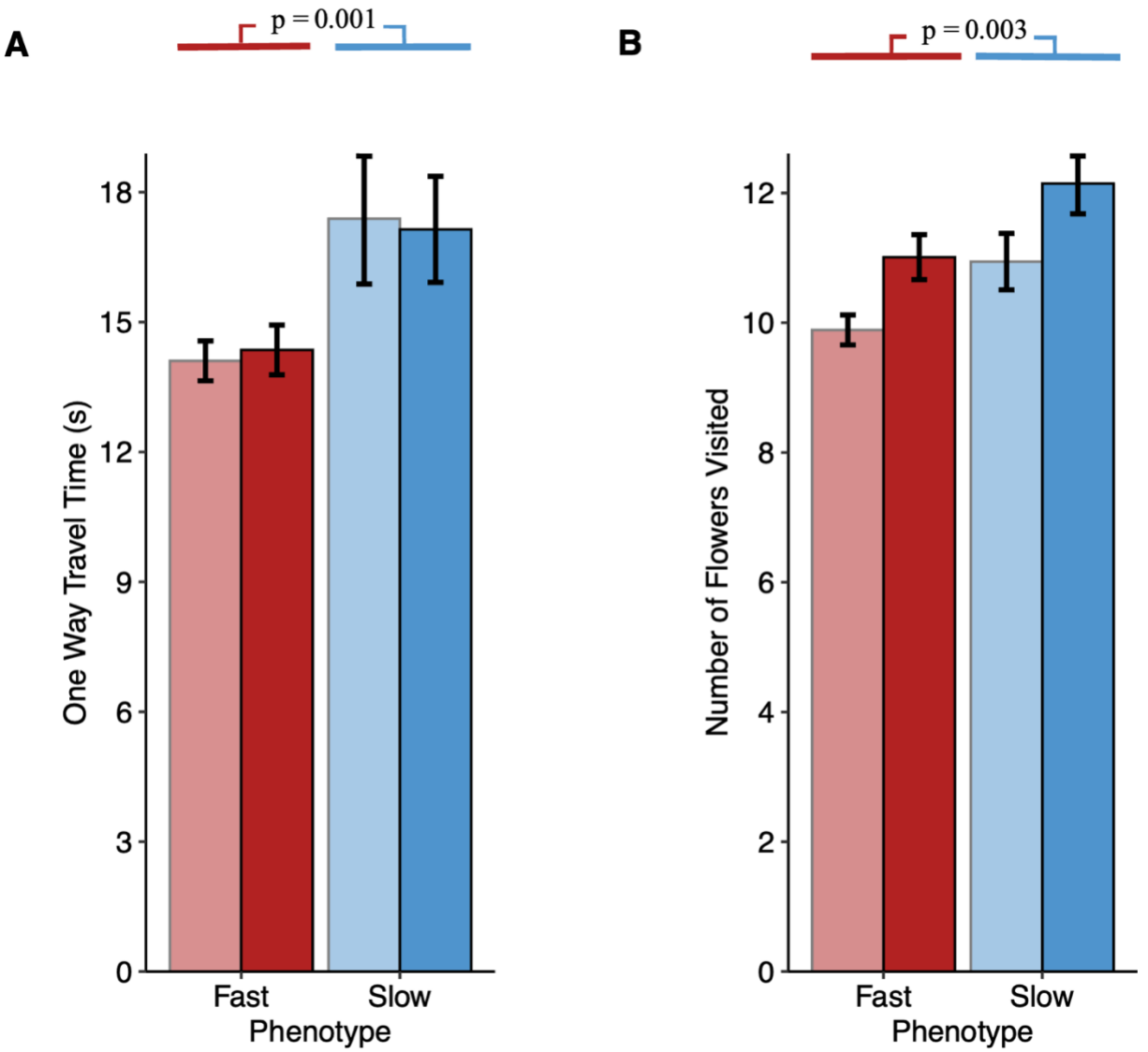


Figure 1.2. Foraging differences between Fast and Slow foragers in terms of (A) one-way travel time (n = 449) and (B) number of flowers visited (n = 299). Data represent mean ± s.e with darker bars indicating Low resource conditions and lighter bars indicating High resource conditions. In both figures, the significant effect of phenotype are shown by comparing the red and blue bars while the significance of resource condition is denoted by comparing light vs darker bars.

Table 1.1. Parameter values used for solving the foraging currency model. All parameter values were directly measured in this study except for the one given in italics which was taken from the Schmid-Hempel et al. (1985) paper. The superscripts F and S indicate values for Fast and Slow bees, respectively, while superscripts H and L represent High and Low resource condition respectively. For the measured parameters, means are reported with sample size and 95% confidence intervals.

Parameters	Description	Value	Stats
h	time spent at each flower (handling time)	$11.34^{S/L}$ (s) $16.^{S/H}$ (s) $11.0^{F/L}$ (s) $16.2^{F/H}$ (s)	n = 52; CI (7.38, 15.09) n = 62; CI (10.43, 14.10) n = 100; CI (10.17, 11.86) n = 85; CI (11.26, 13.17)
τ	time spent flying between two successive flowers (interflower time)	$8.32^{S/L}$ (s) $8.56^{S/H}$ (s) $7.48^{F/L}$ (s) $7.71^{F/H}$ (s)	n = 52; CI (6.40, 9.00) n = 62; CI (6.65, 9.91) n = 100; CI (6.95, 8.48) n = 85; CI (6.24, 8.48)
τ_0	travel time from the hive to the arena (one-way travel time)	$17.38^{S/L}$ (s) $16.98^{S/H}$ (s) $14.1^{F/L}$ (s) $14.23^{F/H}$ (s)	n = 99; CI (14.43, 20.34) n = 99; CI (14.74, 19.55) n = 133; CI (13.20, 15.01) n = 118; CI (13.23, 15.48) * p = 0.001
a_0	flight metabolic rate	0.0467^{S} (W) 0.058^{F} (W)	n =14; CI (0.053, 0.063) n = 18; CI (0.036, 0.057) * p = 0.03
a_h	metabolic rate while drinking nectar	0.007^{S} (W) 0.012^{F} (W)	n = 43; CI (0.004, 0.010) n = 35; CI (0.008, 0.016) * p = 0.04
ω	nectar load per flower	1.69^{L} (mg) 3.00^{H} (mg)	
a	*linear increment in metabolic rate with load weight*	*5 x 10^-5 (W/J)*	
e	weight-specific energetic value of sucrose	16.7 (J/mg)	

Foraging Model

We used parameter values measured in the experiment for Fast and Slow foragers (Table 1.1) to solve the foraging model of Schmid-Hempel (1985) for energy gain rate and efficiency. We then calculated a confidence interval for each foraging currency by running 1000 iterations of the model using a simulated gamma distribution for each parameter from which we calculated a mean and a standard error. For all the four treatment groups, the observed number of flowers visited by the two types of foragers closely met the predictions of the efficiency model but not the gain rate model (Table 1.2, Fig 1.3). Both Fast and Slow foragers had higher efficiency under high resource conditions. More importantly, the Slow foragers had a higher efficiency than Fast foragers and were observed to visit a higher number of flowers under both resource conditions.

Table 1.2. Predicted number of flower visits during a single foraging trip in each treatment group, based on the solution of the foraging model for the maximum of each currency, and the observed number of flowers visited with the corresponding currency values for gain and efficiency (with 95% CI). Energetic gain rate is calculated in Watts (joules/second) while energetic efficiency is unitless.

Phenotype/ Resource Condition	**Gain Rate Model**			**Efficiency Model**		
	Predicted N	*Observed N*	*Observed Gain (95% CI)*	*Predicted N*	*Observed N*	*Observed Efficiency (95% CI)*
Slow/Low	60	10.87	1.65 (1.58; 1.72)	9	10.87	40.15 (38.23; 42.07
Fast/Low	60	9.89	1.27 (1.25; 1.29	11	9.89	31.85 (30.97; 32.73
Slow/High	60	12.29	2.30 (2.24; 2.36)	10	12.29	71.21 (70.43; 71.99)
Fast/High	60	11.01	1.14 (2.10; 2.18	10	11.01	54.31 (53.15; 55.47)

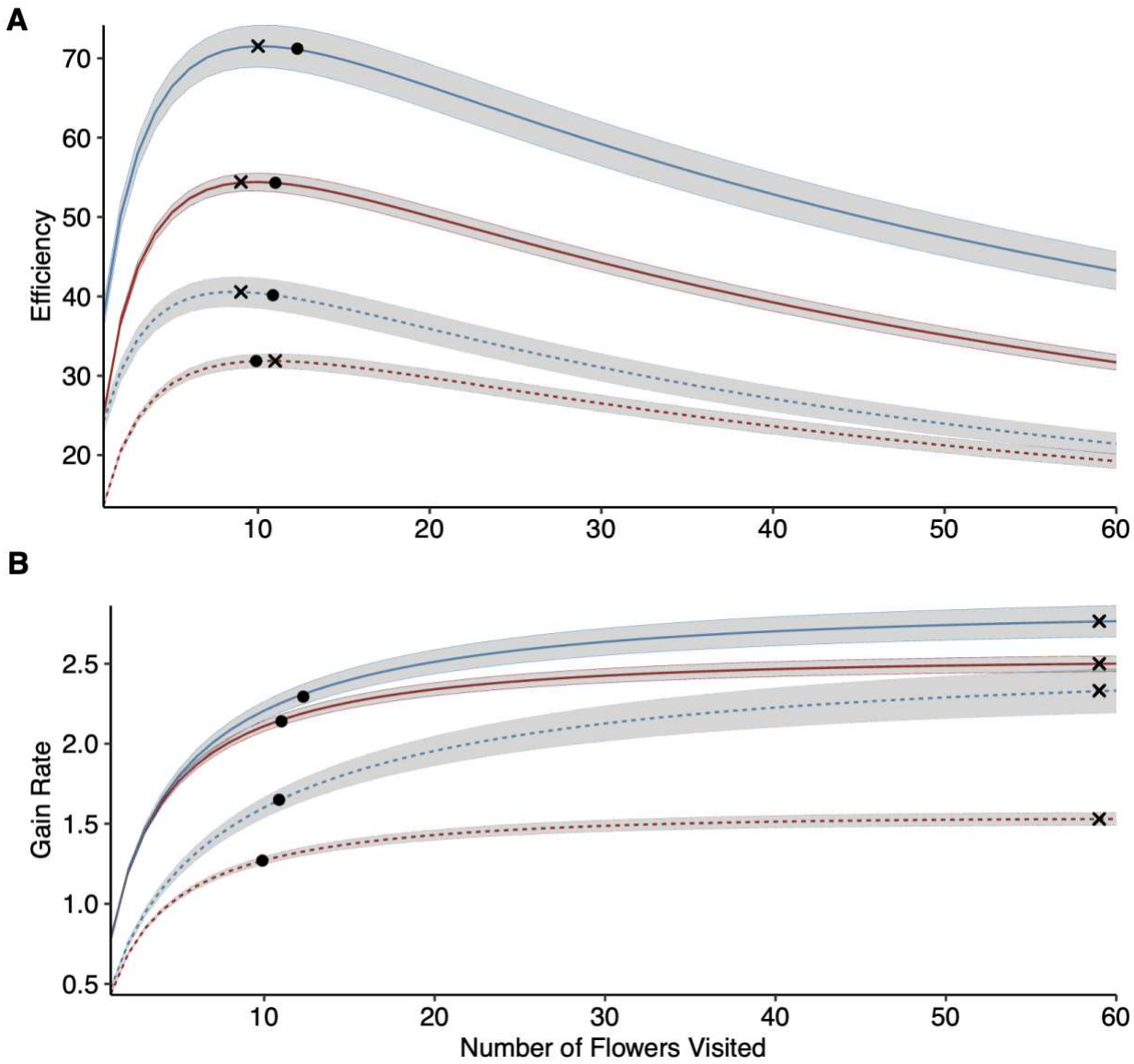


Figure 1.3. Predicted (A) Efficiency, and (B) Gain Rate, plotted as a function of number of flower visits during a foraging trip for each treatment group, calculated from 1000 iterations of the model using a gamma distribution for each parameter value with 95% CI shown as the shaded grey area around each function. Black crosses (**X**) show the predicted number of flower visits based on the maximum of the model solution while black dots (●) denote the observed mean number of flowers visited by each treatment group. Blue and red lines correspond to Slow and Fast foragers respectively, while solid and dotted lines correspond to high and low resource conditions respectively. Energetic efficiency is unitless while Gain Rate is calculated in Watts (joules/second).

Discussion

In this study, to test if differences in metabolic rate translate to differences in foraging strategies that are reflected in foraging gain rate and foraging efficiency, we measured several foraging parameters and solved the optimal foraging model of Schmid-Hempel et al. (1985) for honeybees with low (Slow) and high (Fast) metabolic rate. While Slow and Fast bees did not significantly differ in inter-flower time and handling time, they showed a significant difference in travel time and the number of flowers visited in a single trip. Fast bees took a shorter time to travel to the foraging arena and they visited fewer flowers than Slow bees. Both types of foragers also visited fewer flowers under the low resource condition compared to when it was high, which is not particularly surprising because the energetic gain to cost ratio is likely to be lower for all foragers in a low-value patch. More importantly, our results show that both Slow and Fast bees under both resource conditions seem to maximize their energetic efficiency rather than their rate of energetic gain. At this point, it is important to emphasize that this study was more focused on a relative comparison between the Slow and Fast phenotypes rather than the exact estimations of their foraging currencies. We stress this because we recognize that measuring some of the parameters under more controlled conditions would add to the precision of the quantitative results, but our simulations of the model with a range of values suggest that the model outcomes in terms of the qualitative differences between the two phenotypes are robust to any such modifications.

Energetic considerations are important in foraging decisions because even though foragers can gain more energy by visiting more resources, it comes with an increasing cost which results in a decrease in the net energetic gain as costs starts outweighing benefits. The net energetic gain (G-C) is lower for the Fast bees due to their higher foraging costs, driven largely by their higher metabolic rate. The observation that both types of foragers maximize efficiency rather than gain

rate is consistent with the hypothesis that honeybees, by maximizing their energetic efficiency while foraging, can extend their foraging lifespan and overall lifetime contribution to the colony (Schmid-Hempel et al. 1985). The influence of foraging effort on survival and lifespan is likely to be an important selective force for the evolution of life-history traits at both the individual and the colony level. This is supported by the observation that the lifespan of honeybee workers has heritable components (Kulinčević and Rothenbuhler 1982; Rueppell et al. 2007). The observed variation in lifespan consists of an intrinsic component related to ageing due to physical and functional senescence and an extrinsic component related to mortality risk due to environmental hazards encountered while foraging (Neukirch 1982; Visscher and Dukas 1997; Amdam and Omholt 2002; Margotta et al. 2018). A relationship between metabolic rate and foraging effort can influence both these components of lifespan and future studies need to separate them to fully resolve this link.

When comparing the energetic efficiency between the two metabolic rate phenotypes, we find that although Slow foragers are always more efficient than Fast foragers, this difference between the two is larger under high resource conditions than under low resource conditions (Table 1.2). The Slow foragers seem to hold an advantage over the Fast foragers even if we consider gain rate as the currency. These results seem to challenge both theoretical and empirical work that suggest a higher metabolic rate to be adaptive in resource rich environments and an advantage to low metabolic rate individuals in resource poor conditions (Biro and Stamps 2010; Burton et al., 2011; Auer et al. 2015; Katz and Naug 2020). Furthermore, the fact that Slow foragers outperformed Fast foragers in both conditions leads to questions regarding why the two phenotypes are maintained in the population. In order to address this seeming paradox, we turned to the

predictive power of our foraging model and ran hypothetical scenarios to gain insights into possible advantages to high metabolic rate foragers. Since the energetic costs of travel represent the major foraging cost and the largest difference between the two metabolic rate phenotypes is that the Fast bees have a faster travel time, allowing them to travel to and from the foraging arena ~1.2 times faster than the Slow bees (Fig 1.2A), we magnified this difference and found that an even higher difference in this regard can lead to a higher efficiency for the Fast foragers (Fig. 1.4). Ecologically, a higher difference in travel time can result from two different mechanisms, (1) Fast bees preferentially visiting patches closer to the colony while Slow bees forage farther, and/or (2) Fast bees traveling to a natural food patch at a larger distance even faster than what we observed in our experiment. Since the foraging arena was located at a fixed distance in our experiment, we cannot resolve between these two possibilities and further work would be needed to answer this question. Since high metabolic rate bees also have higher consumption rates (Mugel and Naug 2020), a possible hypothesis that might explain their lower efficiency is the idea that efficiency maximization works well only when self-feeding rates are low (Ydenberg et al. 1994).

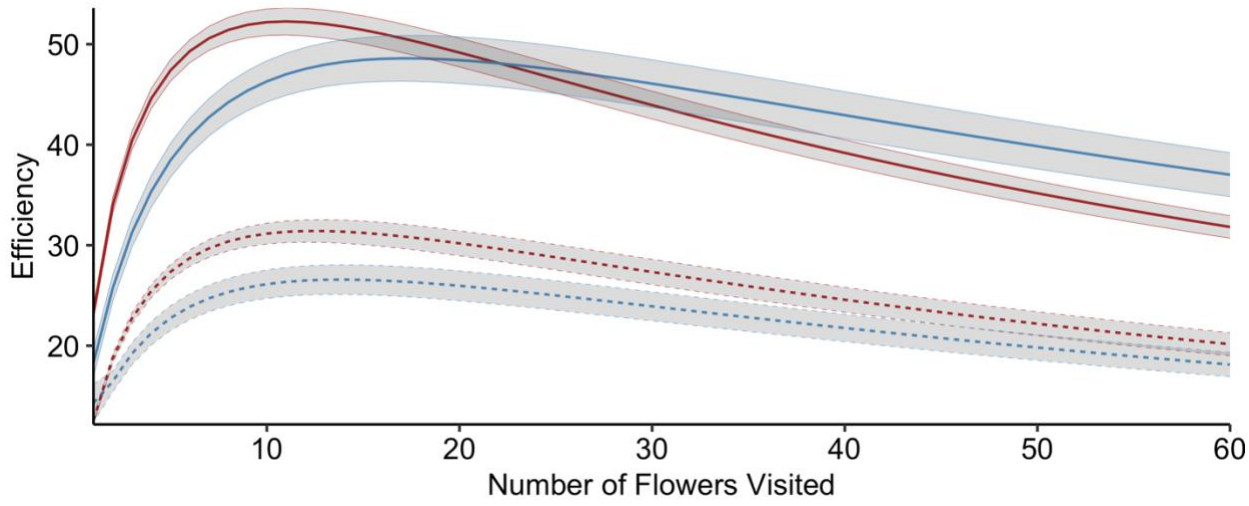


Figure 1.4. Foraging efficiency of Fast and Slow foragers with hypothetical values that magnify the difference between them in terms of one-way travel time (τ_0) such that $\tau_{0\,Slow} = 2 \times \tau_{0\,Fast}$.

Blue and red lines correspond to Slow and Fast foragers respectively, while solid and dotted lines correspond to high and low resource conditions respectively.

In terms of their foraging contribution to the colony, what might also compensate for the lower efficiency of high metabolic rate bees on a single foraging trip is that they may have the capacity for a higher overall foraging rate (Harrison and Fewell 2002). High metabolic rate foragers also have a higher aerodynamic capacity that might make them better equipped for carrying heavier and externally carried loads such as pollen (Feuerbacher et al. 2003), contributing to a division of labor between the two phenotypes in terms of foraging resource. If Fast bees are indeed more likely to contribute to pollen resources in the colony while Slow bees contribute to nectar resources, this extrapolates to saying that Fast bees will contribute more to colony growth while Slow bees contribute more to colony survival. This is supported by the observation that colonies at lower latitudes have a higher frequency of Fast MDH alleles (Nielsen et al. 1994; del Lama et al. 2004) and also show higher rates of growth and reproduction compared to colonies at higher latitudes. The latitudinal gradient of the MDH alleles and its influence on foraging and resource acquisition patterns show how metabolic rate might be fundamentally connected to life history differences across latitudes. High metabolic rate Fast bees also seem to make a higher contribution to other aspects of colony division of labor such as thermoregulation (Mugel and Naug *In press*) and further studies are required to understand how the colony might derive an adaptive advantage from maintaining a metabolic diversity.

The broad goal of this study was to understand the possible links between energetics and foraging behavior and how it might mediate a link between metabolic rate and life history, given its role in resource acquisition. The implications of this link are further complicated in a group-

living animal such as the honeybee colony in which foraging is a collective process that is an emergent outcome of differences in foraging behavior expressed at the individual level. Since metabolic rate shapes the energetic cost of foraging, it is important to understand how the inter-individual variation in metabolic rate within a colony might shape its resource acquisition patterns. In our experiment, low metabolic rate Slow foragers showed a higher efficiency than high metabolic rate Fast foragers, a difference that was even more exaggerated under high resource conditions. Although this may seem contradictory to the expectation based on similar studies on foraging, we offer some possible explanations for it. In addition to the possibility that high metabolic rate bees contribute to the colony in a different fashion, it needs to be pointed out that our study implemented a common garden design that might reduce the difference between the two metabolic rate phenotypes if their performance is shaped by gene x environment interactions. Temperature is known to have a significant influence on metabolic rate which might lead to contrasting performances of each phenotype at different temperatures (latitudes). In fact, temperature is proposed as a major selective force that mediates the latitudinal gradient of these Slow-Fast MDH phenotypes (Hatty and Oldroyd 1999). It has been pointed out that the performance of slow-fast phenotypes can be subject to complex influence from numerous factors such as habitat complexity, spatial and temporal variability of resources, competition, etc. (Réale et al. 2010). This makes it challenging to decipher how metabolic rate shapes resource acquisition and thereby life history traits and future studies on this topic would need to consider such ecological factors more explicitly, a daunting challenge by all means. Interspecific comparisons between species with different foraging ecologies and life histories (Charlton and Houston 2010) might offer important insights in this regard.

CHAPTER 2: HOW DOES METABOLIC RATE INFLUENCE BEHAVIORAL AND LIFE HISTORY PARAMETERS IN HONEYBEES

Introduction

The Pace of Life Hypothesis, extending the rate of living hypothesis, seeks to functionally integrate metabolic theory with life history theory by assigning individuals on a slow – fast axis such that individuals with a lower metabolic rate have slower behavioral and life history traits than those with a higher metabolic rate (Ricklefs and Wikelski 2002, Réale et al. 2010). This link between metabolic rate and life history traits is mediated through foraging behavior since it drives resource acquisition and is strongly influenced by differences in metabolic rate (Biro and Stamps 2010). Individuals with a higher metabolic rate are proposed to have the ability to acquire resources at a faster rate that might allow them to fuel growth and reproduction although the higher activity comes with a higher cost of living in terms of mortality risk that comes from external risks such as predation and other environmental hazards and internal processes that lead to oxidative damage (Rubner, 1908; Pearl, 1928). This idea has however seen mixed empirical support and has therefore been extensively debated without any clear consensus (Speakman and Selman, 2011).

The hypothesized negative relationship between metabolic rate and lifespan sees overall stronger support in ectotherms such as in *C. elegans* (Van Voorhies and Ward 1999) and crickets (Okada et al., 2011). In contrast, many studies with endotherms such as hamsters (Oklejewicz and Daan, 2002), dogs (Speakman et al., 2003), mice (Speakman et al., 2004), birds (Holmes and Austad 1994; Holmes et al. 2001; Furness and Speakman 2008) have reported a positive relationship between the two. It gets even more confusing as a similar positive relationship has

also been found in ectotherms such as butterflies (Niitepõld and Hanski, 2013) as well as the lack of any relationship in model species such as *Drosophila* (Melvin et al. 2007). More recent analyses of this hypothesis have therefore emphasized that the relationship between metabolic rate and lifespan is highly complex and a greater need for intraspecific studies (Speakman et al. 2005).

In social insects such as the honeybee, *Apis mellifera*, individual differences in metabolic rate and its correlation with behavioral and life history traits are likely to have an emergent effect at the colony level. In a honeybee colony, there is a significant amount of natural variation in both foraging activity and lifespan and one of the factors that is known to explain this large variation is the timing of ontogenetic shift in the lifetime of a forager (Rueppell et al. 2007). Honeybees demonstrate temporal polyethism such that they spend the first part of their life performing in-hive tasks and then transition to performing foraging tasks outside the hive in the latter part of their life (Seeley 1982). The ontogenetic shift between these two life stages is known as the Age at Onset of Foraging (AOF) and several studies have shown that an accelerated behavioral development resulting in an advancement in this shift can increase functional senescence and mortality rates (Visscher and Dukas, 1997; Dukas, 2008, Perry et al 2015). A recent study demonstrated a negative relationship between metabolic rate and these critical life history parameters such as AOF and lifespan in honeybees (Mugel and Naug 2020), but this study was somewhat limited in its scope because it measured AOF through behavioral sampling, which limits the resolution of this sensitive parameter. Additionally, lifespan of individuals was measured while being restricted in cages, which disconnects it from the important impact of external mortality risk.

Some recent studies have made use of Radio Frequency Identification technology to estimate time-activity budgets and calculate important life history parameters such as AOF and lifespan in honeybees (Requier et al 2020; Prado et al. 2020). This technology has allowed researchers to remotely record all foraging trips made by a tagged individual, which enables the calculation of various behavioral and life history parameters at a high resolution for large sample sizes that can be used in predictive models (Perry et al 2015; Require et al. 2020). By integrating RFID technology and respirometry to measure metabolic rate with genetic lines of bees bred for low and high metabolic rate, in this study we aim to provide a comprehensive test of the proposed relationships between metabolic rate, foraging behavior, and life history. We test the predictions of the 'rate of living' hypothesis that bees with higher metabolic rates show earlier ontogenetic shifts, increased foraging activity and shorter lifespan.

Methods

Experimental design

We conducted two experiments, one with wild-type unselected bees and another with bees from genetic lines selected for low (Slow bees) and high (Fast bees) metabolic rate. In the first experiment with wild-type bees, we measured the metabolic rate of individuals at two different life stages and investigated its influence on behavioral and life history parameters using automated Radio Frequency Identification (RFID) monitoring. The second experiment, using RFID technology to monitor foraging behavior and life history with Slow and Fast bees, allowed us to experimentally test the effects of metabolic rate on behavioral and life history parameters while controlling for natural variation in metabolic rate phenotypes that that is greater among individuals in a wildtype population.

Colony setup

For each experiment, brood combs were extracted from their respective source colonies (Fast or Slow), hatched in an incubator set to 32 °C and newly emerged bees were introduced into an experimental colony at biweekly intervals, creating a common garden design that controlled for any colony level social effects on foraging behavior.

Measurement of behavioral and life history parameters

Bees to be introduced into the experimental colony were fastened with individual number tags and Radio Frequency Identification (RFID) tags (Microsensys mic3 tags) on their thorax. The colony was connected to the outside with a tunnel and two RFID readers were placed at consecutive parts of the tunnel automatically recorded a tagged bee as it passed through the readers. Depending upon which reader records a given bee first and which one records it second, one can sort these records into departures and returns during foraging.

These raw scan records were first processed with the *feedr* R package to filter out duplicate recordings (LaZerte, 2017). The filtered dataset was then processed with "Track-a-Forager", a Java program that uses an algorithm to assemble the raw data of scans into complete foraging trips (van Geystelen et al 2016). Over both experiments, of the 588 RFID tagged bees introduced into the experimental hive, 342 were recorded leaving the hive at least once. The Track-a-Forager software was then used to calculate the daily and total number of foraging trips as well as the length of each trip for each bee. The *aof* R package, which utilizes a behavioral change point analysis approach, was used to detect the age at onset of foraging (AOF) from the records of the first time each bee

left the hive on a foraging trip (Requier et al. 2020). Lifespan of a bee was calculated using the last date it was recorded and its total number of foraging days was calculated as the number of days alive after AOF.

Metabolic rate measurement

In the experiment with wild-type bees, a same tagged individual was collected from the experimental hive when it was 1 week old and once again when it was 3 weeks old. Each bee was weighed, and its Routine Metabolic Rate (RMR) was measured using flow through respirometry that consisted of a FoxBox with a multiplexer unit (Sable Systems) that allowed the simultaneous measurement of seven bees at a time. Each bee was placed in a clear 250 mL sealed plastic chamber and ambient air scrubbed of H_2O and CO_2 was passed through the chamber at a constant rate of 500 mL/min and the CO_2 concentration in the excurrent airflow was recorded every second and corrected for drift by subtracting baseline CO_2 readings taken prior to each recording. RMR was calculated as the mean weight corrected CO_2 production (mL hr^{-1} g^{-1}), which was then transformed into a power output (W g^{-1}) by multiplying it by 21.4 J mL^{-1} CO_2 and dividing by 3600 J hr^{-1} (Feuerbacher et al. 2003, Mugel and Naug 2020).

Statistical analysis

From the first experiment with wild-type bees, the repeatability (R) of metabolic rate was calculated, using the two measurements of metabolic rate for a given bee across a temporal context, with a linear mixed effects model approach with parametric bootstrapping (n = 1000) using the *rptR* R package (Stoffel 2017). The association between RMR and behavioral/life history traits were analyzed with pairwise Pearson product-moment correlations using the *stats* R package (R

Core Team, 2021). The data from the second experiment was analyzed with General Linear Models to test the effect of phenotype on behavioral and life history traits using the *car* R package (John Fox and Sanford Weisberg 2019). For each dependent variable, models were first run with phenotype and date of birth as predictive variables including the interaction between the two. If the interaction was found to be non-significant, it was then dropped from the model to increase the power of each predictive variable. All statistical analyses were performed in R (version 4.0.5, R core team).

Results

Routine Metabolic Rate

We measured Routine Metabolic Rate of a bee at two biologically significant stages – when it was 1 week old and of pre foraging age and when it was 3 weeks old and of foraging age. The repeatability of RMR between Week 1 and Week 3 was low (R = 0.05, p = 0.32, n = 15). Overall, RMR measurements were significantly different across Week 1 and Week 3 (Week 1: 28.19 ± 16.64 mW/g, Week 3: 51.72 ± 21.86, Paired t-Test: $t_{28} = 3.78$, $p = 0.003$; Fig 2.1a). A pairwise Pearson's product-moment correlation found no significant correlation between the metabolic rate measured at Week 1 and Week 3 ($r = 0.13$, $p = 0.26$, Fig. 2.1b).

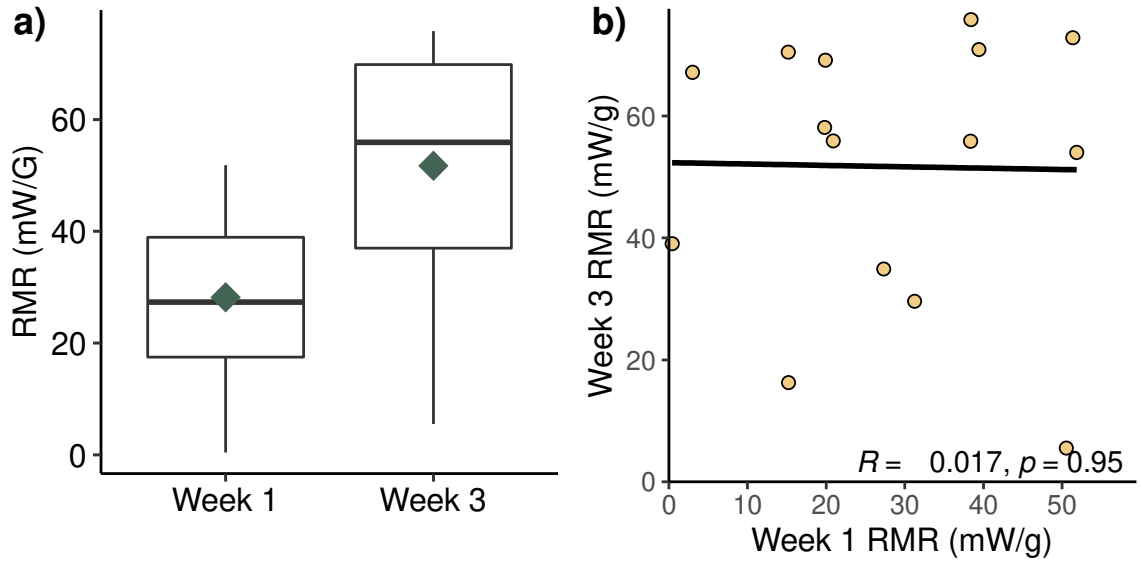


Figure 2.1. Metabolic rate of individual bees measured when they were 1 and 3 weeks old with the data plotted as (a) boxplots with the mean represented by green diamonds, and (b) scatterplot with dots representing individual bees and the solid line showing the direction of the Pearson product moment correlation.

Behavior and life history parameters in wild-type bees

The behavioral change point analysis calculated the Age at Onset of Foraging (AOF) for 23 bees and the following analysis was performed for only these bees that were confirmed foragers. A pairwise Pearson's product-moment correlations analysis found significant correlations between AOF and lifespan ($r = 0.97$; $p < 0.001$; $n = 22$, Fig 2.2a), the number of foraging days and lifespan ($r = 0.414$; $p = 0.04$; $n = 22$, Fig 2.2b), and the number of foraging days and average trip length ($r = 0.625$; $p = 0.001$; $n = 22$, Fig 2.2c). The remaining pairwise comparisons were not significant (Table S2.1).

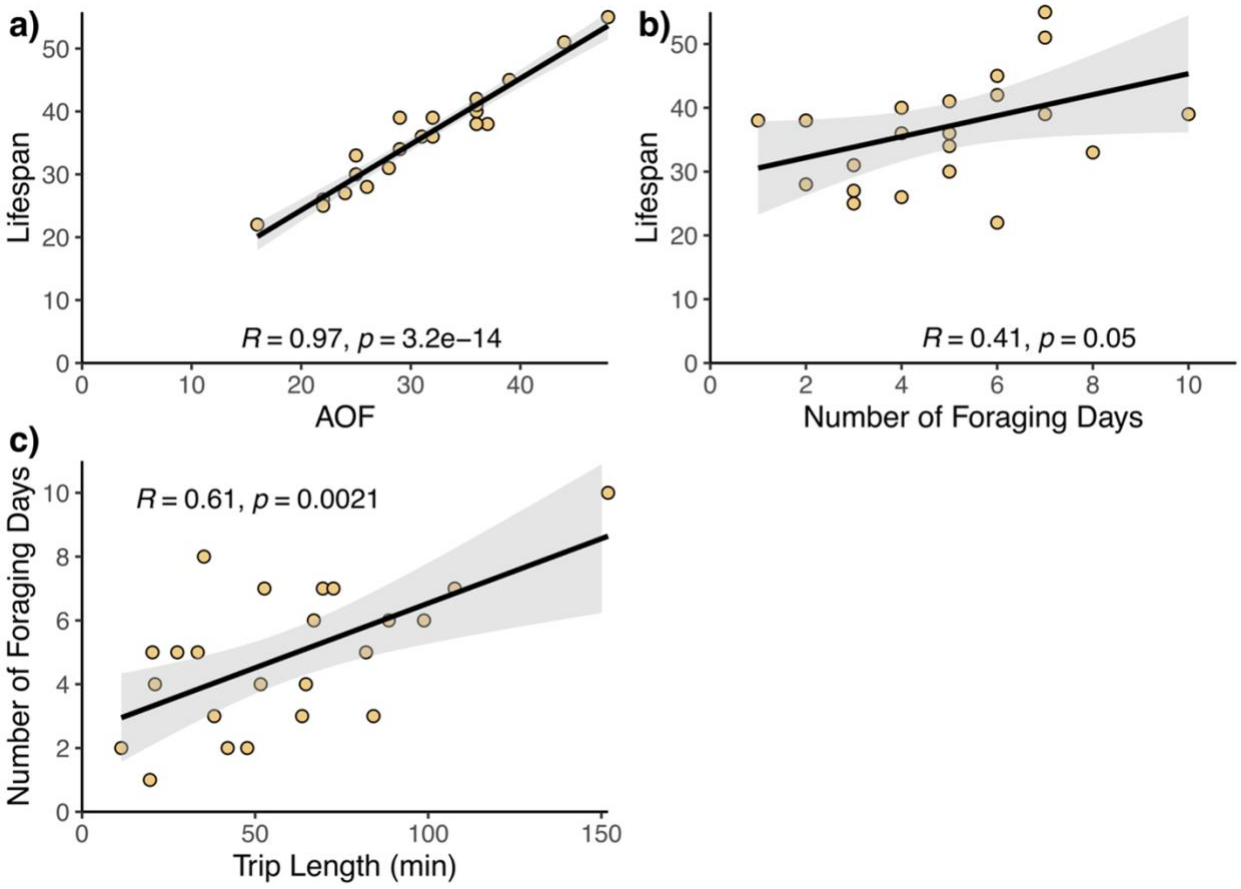


Figure 2.2. Pairwise correlations between the different behavioral and life history parameters with colored dots representing individual bees and the sold lines showing the direction of correlation with the grey area depicting their confidence intervals.

We then analyzed the only 15 individuals for which we were able to measure all the life history and behavioral parameters and week 1 and week 3 metabolic rates. A pairwise Pearson's product-moment correlation analysis revealed a significant negative correlation of Week 1 RMR with both lifespan (r = -0.52; p = 0.027; n = 15, Fig 2.3a) and AOF (r = -0.543; p = 0.016; n = 15, Fig 3b). However, Week 3 RMR did not significantly correlate with either lifespan (r = 0.065; p = 0.78; n = 15, Fig 2.3c) or AOF (r = 0.15; p = 0.52; n = 15, Fig 2.3d). No other behavior or life history parameters showed a significant correlation with metabolic rate (Table S1).

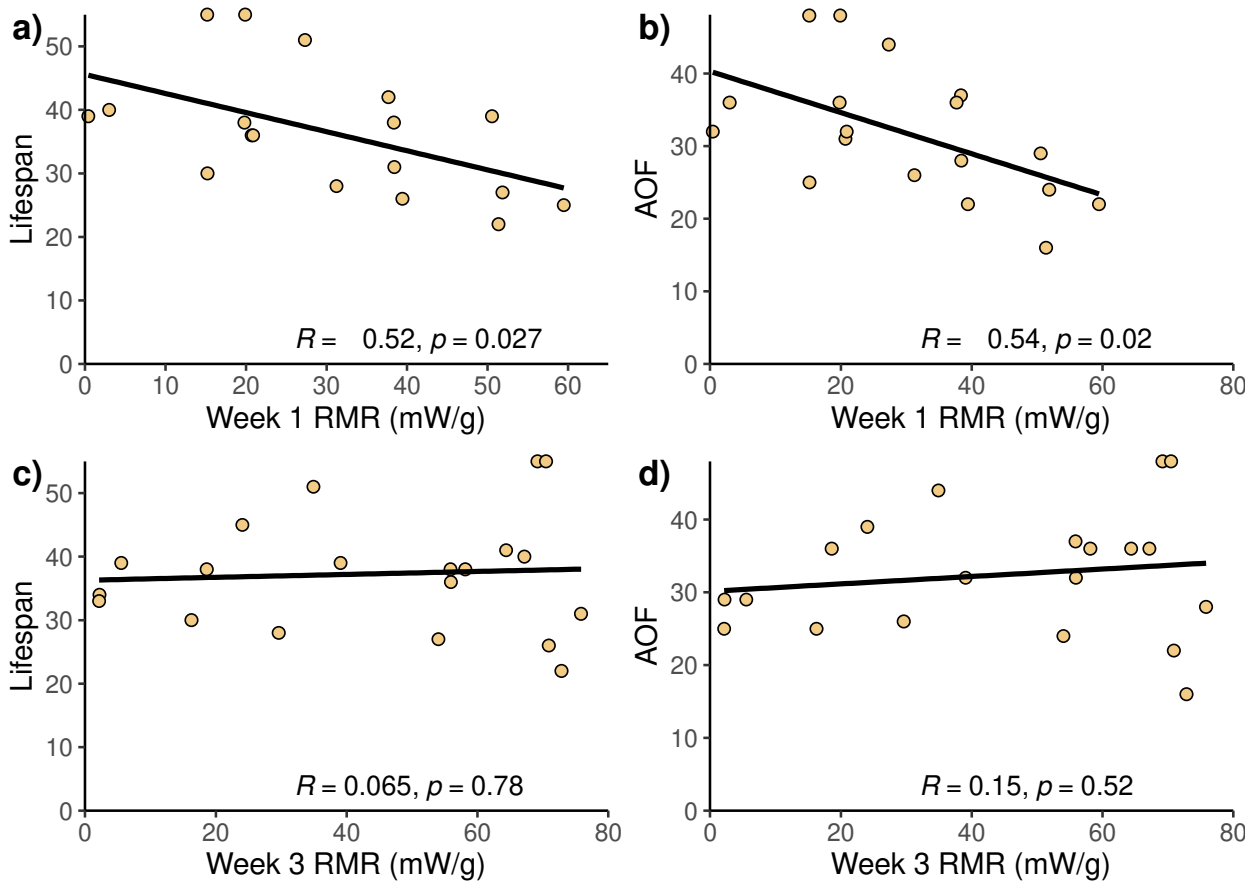


Figure 2.3. Pairwise correlations between the different life history parameters and metabolic rate measurements with colored dots representing individual bees and the sold line showing the direction of correlation and grey areas representing 95 percent confidence intervals.

Behavioral/life history parameters of metabolic rate phenotypes

Among the individuals from the phenotypic lines (Fast and Slow), there was a significant influence of both phenotype (2-way ANOVA: $F_{1,83} = 4.57$, $p = 0.03$) and birthdate ($F_{8,83} = 3.45$, $p = 0.002$) on the lifespan of a bee. Post hoc analysis showed that Fast bees had an average lifespan of 29.15 days, which was significantly higher than Slow bees which lived for 24.84 days ($t_{83} = 2.33$, $p = 0.022$, Fig. 2.4a).

There was a significant interaction effect between phenotype and birthdate ($F_{1,83} = 4.31$, $p = 0.04$) as well as significant independent effects of both Phenotype ($F_{1,83} = 4.95$, $p = 0.02$) and birthdate ($F_{1,83} = 14.37$, $p < 0.001$) on AOF. Fast bees had a significantly later AOF of 23.63 days than Slow bees which had an AOF of 19.75 days ($t_{83} = 2.56$, $p = 0.01$, Fig. 2.4b).

There was a significant independent effect of birthdate ($F_{1,83} = 5.74$, $p < 0.001$) but no significant effect of phenotype ($F_{1,83} = 0.38$, $p = 0.55$) on daily trip frequency (Fig 2.4c). Fast bees averaged 1.55 trips per day while Slow bees averaged 1.56 trips per day, a non-significant difference (2 sample t test: $t_{83} = 0.047$, $p = 0.96$).

There was no significant effect of phenotype ($F_{1,83} = 1.76$, $p = 0.19$) or birthdate ($F_{1,83} = 0.64$, $p = 0.71$) on trip length. Fast and Slow bees did not significantly differ in the average time spent on each foraging trip with Fast bees averaging 47.07 minutes per trip and Slow bees averaging 38.30 minutes per trip (2 sample t-test: $t_{1,83} = 1.41$, $p = 0.16$, Fig. 2.4d).

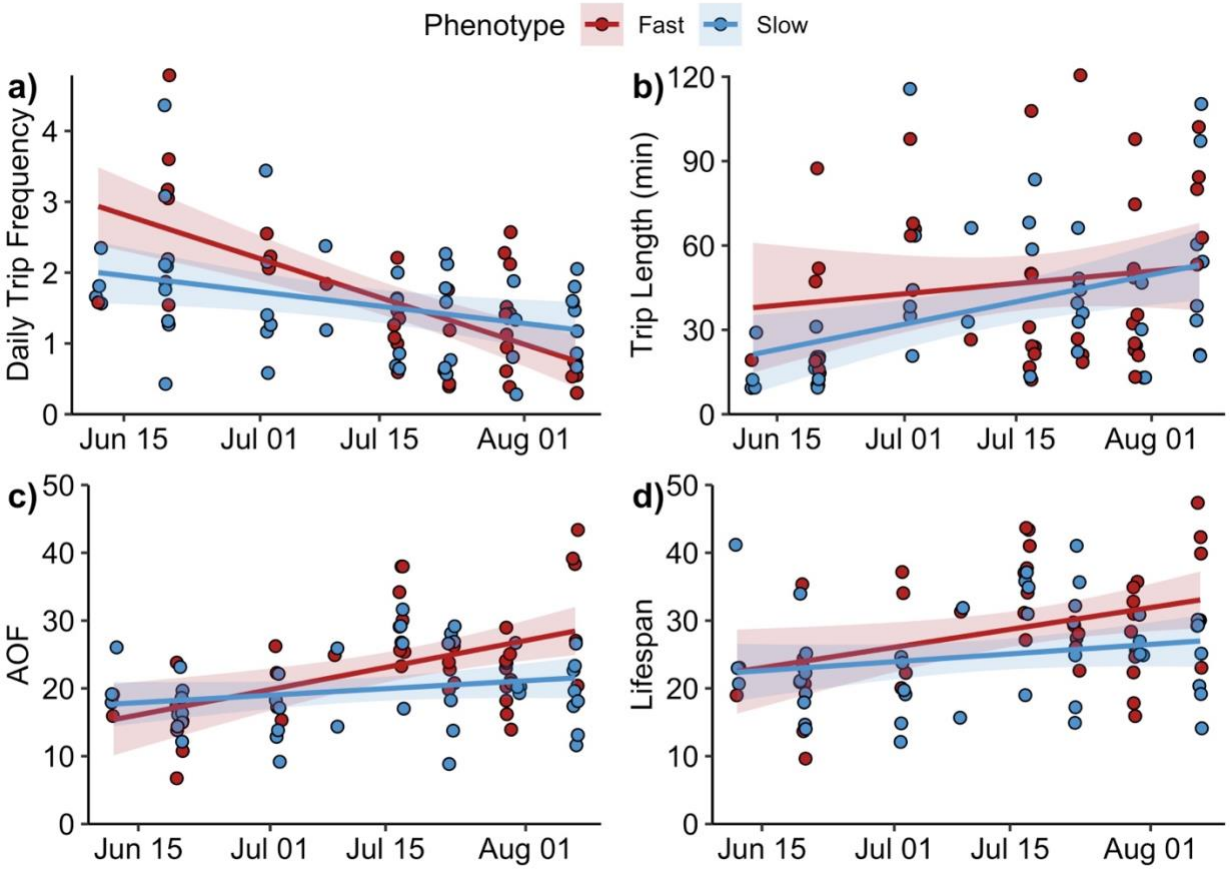


Figure 2.4. Behavioral and life history parameters of Slow (blue) and Fast (red) bees in terms of (a) daily foraging rate, (b) trip length, (c) age at onset of foraging, and (d) lifespan, plotted on a temporal axis representing the date of birth of each bee. Dots represent individual bees and the lines represent the fitted regression lines.

DISCUSSION

In this study, we aimed to comprehensively test the 'rate of living' hypothesis by exploring the relationships between metabolic rate, foraging behavior and life history traits in honeybees using both wild-type and selected lines of bees with low and high metabolic rates. Since metabolic rate is the key parameter in this hypothesis, we first tested its repeatability which is defined as the among-individual fraction of the total variance of a trait in a population and indicates consistent phenotypic differences among individuals as against plasticity in the trait (Biro and Stamps 2016). We found the repeatability of metabolic rate to be quite low across the ontogeny of bees as they

transition from pre-foraging to foraging tasks, which suggests considerable plasticity in this trait. These results are contrary to a meta-analysis which found metabolic rate to be considerably repeatable across a wide variety of taxa (Nespolo and Franco 2007). Aside from the fact that this meta-analysis is heavily skewed toward vertebrates, in order to reconcile our findings of the low repeatability of metabolic rate in honeybees, they need to be viewed in the context of temporal polyethism – a significant ontogenetic shift in which honeybee workers shift from performing in hive tasks such as brood care to much more energy intensive out of hive tasks like foraging. We found metabolic rate of 1-week-old bees to be significantly lower than 3-week-old adult bees, which is consistent with previous findings (Schippers et al. 2010; Harrison 1994). It is important to point out that these previous studies only looked at metabolic rate in groups of young and old bees rather than across the ontogeny of single individuals. To the best of our knowledge, our study therefore is the first to measure the repeatability of metabolic rate in honeybees. The low repeatability of metabolic rate observed here could result from differences in foraging experience which is known to have a significant influence on metabolic rate in honeybees. Some other studies which have shown high repeatability of metabolic rate in insects (Niitepõld & Hanski, 2013, Darveau et al. 2014) however measured the trait across short time intervals of a couple of days and therefore does not account for any effects of ontogeny or experience.

To fully understand the association between metabolic rate and life history traits in honeybees, it is therefore important to fully account for the difference among individuals in their foraging behavior. In our study with wild-type bees, while the two important life history traits, AOF and lifespan were negatively correlated with metabolic rate in the pre-foraging stage as would be predicted by the pace of life hypothesis (Mugel and Naug 2020), none of the foraging

parameters such as trip frequency or trip time was however correlated with metabolic rate. There was a strong positive correlation between the two important life history parameters, AOF and lifespan, a finding that is consistent with previous studies (Rueppell et al. 2007, Mugel and Naug 2020). Bees transitioning to foraging later in life lived longer because this presumably reduced their exposure to both extrinsic and intrinsic factors that are associated with mortality. While this might seem contradictory to the result that lifespan is also positively correlated with the number of days a bee spends foraging and trip length, it could be an outcome of the presence of "elite" foragers which perform the majority of the colony foraging activity (Tenczar et al. 2014). The somewhat mixed patterns of association between life history traits and metabolic rate observed here is consistent with similar observations across different taxa and emphasizes the need for more rigorous studies that can experimentally control for the effects of metabolic rate.

The metabolic rate phenotypic lines consisting of Fast and Slow bees allow a more direct test of the rate of the 'rate of living' hypothesis, which would predict Fast bees to live fast and die young compared to Slow bees. While once again there was no effect of metabolic rate phenotype on behavioral parameters such as foraging rate and trip length, it had a significant effect on the two life history traits although in a manner that is contradictory to the results from the previous experiment and the Rate of Living hypothesis, with Fast bees showing a later AOF and a longer lifespan. However, it is important to note that in our study, birthdate of foragers was a significant predictor of lifespan, AOF and daily foraging rate. Foraging rate declined as the season progressed from early to late summer and coincided with an increase in AOF and lifespan. The shift in these three traits is consistent with the known change in the resource environment and the colony lifecycle over the season (Seeley 1995). In early summer, bees transition to foraging early to take

advantage of the abundant resource availability, foraging at a higher rate to harvest these resources and presumably paying a cost that is expressed in a shorter lifespan.

It is also important to notice that the difference in behavioral and life history traits across this temporal scale is more prominent in the Fast bees. This suggests that the change in resource availability, which drives this shift, has a stronger influence on Fast bees with a higher metabolic rate. This is especially significant for the age at onset of foraging, seen as a significant interaction effect between birthdate and metabolic rate phenotype. Previous work with these metabolic rate phenotypes showed that Slow bees visit more flowers on a single foraging trip and are more energetically efficient than Fast bees under both high and low resource conditions (Cassano and Naug, In review). Therefore, one can speculate that the lower effect of seasonal differences on the Slow bees could be due to their higher overall efficiency, which allows them to perform equally well under a changing resource environment. Fast bees seem to have a lower foraging rate later in the season when resource availability is low, which also coincides with a later AOF and longer lifespan. These patterns also show that the effect of metabolic rate on behavior and life history is likely to be strongly influenced by genotype x environment interactions, emphasizing the complex nature of this relationship.

Although a changing resource availability might partly explain the behavioral and life history differences between Slow and Fast bees across the season, it is still somewhat surprising that the Fast bees with a higher metabolic rate had an overall later AOF and a shorter lifespan, both of which stand in contrast with earlier studies (Mugel and Naug 2020) and the Rate of Living hypothesis. Lately, tests for this hypothesis have focused on the Reactive Oxygen Species (ROS)

hypothesis of aging which assumes that higher energy use and accompanying increased oxygen consumption can lead to increased levels of oxidative stress that leads to shorten lifespan in some species (Hulbert et al. 2007). However, some studies also show that individuals or species with higher metabolic rates have higher levels of antioxidants that can counter such oxidative stress (Salin et al. 2015). In honeybees it has been shown that more active individuals show higher levels of oxidative damage but not higher levels of antioxidants, an imbalance which can lead to cellular damage and shorter lifespan (Margotta et al. 2018). Although there was no effect of metabolic rate phenotype on behavioral traits such as daily trip frequency and average trip length in our study, the next step would be to test for such markers of physiological damage in these foragers with different metabolic phenotypes and experimentally manipulated activity levels.

Overall, our study found that the rate of living hypothesis is not clearly supported in honeybees but did shed light into the relationship between metabolic rate, behavior, and life history. It sets the framework for further investigations into these relationships and future research should focus on experiments with large samples sizes and changes in resource availability across the colony cycle. Understanding these relationships will allow a unique perspective into how individual level physiology, behavior and life history traits scale to determine fitness and performance at the group level. Such an understanding can also have economic implications as honeybees are a key species that provides pollination services in agriculture around the world.

REFERENCES

Abrams P. 1983 Life-history strategies of optimal foragers. Theor. Popul. Biol. 24, 22-38.

Amdam GV, Omholt SW. 2002 The regulatory anatomy of honeybee lifespan. J. Theor. Biol. 216, 209-228.

Becerra-Guzmán, F., Guzmán-Novoa, E., Correa-Benítez, A., & Zozaya-Rubio, A. 2005. Length of life, age at first foraging and foraging life of africanized and European honey bee (apis mellifera) workers, during conditions of resource abundance. Journal of Apicultural Research, 44(4), 151–156.

Biro PA, Stamps JA. 2010 Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? Trends Ecol. Evol. 25, 653-659.

Brown JH, Gillooly JF, Allen AP, Savage VM, West GB. 2004 Toward a metabolic theory of ecology. Ecology 85, 1771-1789.

Careau V, Garland T. 2012 Performance, personality, and energetics: Correlation, causation, and mechanism. Physiol. Biochem. Zool. 85, 543-571.

Cassano J & Naug D. Metabolic rate shapes differences in foraging efficiency among honeybee foragers. In review.

Charlton NL, Houston AI. 2010 What currency do bumble bees maximize? PLoS ONE 5, e12186.

Charnov, E.L. 1976. Optimal foraging: The marginal value theorem. Theor. Popul. Biol. 9, 129-136.

Chang W, Cheng J, Allaire JJ, Sievert C, Schloerke B, Xie Y, Allen J, McPherson J, Dipert A, Borges B. 2021. shiny: Web Application Framework for R. R package version 1.6.0.

Darveau, C. A., Billardon, F., & Belanger, K. 2014. Intraspecific variation in flight metabolic rate in the bumblebee Bombus impatiens: Repeatability and functional determinants in workers and drones. Journal of Experimental Biology, 217(4), 536–544.

Decourtye, A., Devillers, J., Aupinel, P., Brun, F., Bagnis, C., Fourrier, J., & Gauthier, M. 2011. Honeybee tracking with microchips: A new methodology to measure the effects of pesticides. Ecotoxicology, 20(2), 429–437.

Del Lama MA, Oliveira Souza R, Andréa Araneda Durán X, Espencer Egea Soares A. 2004 Clinal variation and selection on MDH allozymes in honeybees in Chile. Hereditas 140, 149-153.

Dukas, R. 2008. Life History of Learning: Performance Curves of Honeybees in the Wild. Ethology, 114(12), 1195–1200.

Feuerbacher E, Fewell JH, Roberts SP, Smith EF, Harrison JF. 2003 Effects of load type (pollen or nectar) and load mass on hovering metabolic rate and mechanical power output in the honey bee *Apis mellifera*. *J. Exp. Biol.* **206**, 1855-1865.

Harper, D. G. C. 1994. Some comments on the repeatability of measurements. Ringing and Migration, 15(2), 84–90.

Harrison JF, Fewell JH. 2002 Environmental and genetic influences on flight metabolic rate in the honey bee, Apis mellifera. Comp. Biochem. Physiol. A 133, 323-333.

Harrison, J. F., Nielsen, D. I., & Page, R. E. 1996. Malate Dehydrogenase Phenotype, Temperature and Colony Effects on Flight Metabolic Rate in the Malate dehydrogenase phenotype, temperature and colony effects on flight metabolic rate in the Honey-bee, Apis mellifera. Functional Ecology, 10(1), 81-88.

Hatty S, Oldroyd B. 1999 Evidence for temperature-dependent selection for malate dehydrogenase

allele frequencies in honeybee populations. J. Hered. 90, 565-568.

Houston AI. 2010 Evolutionary models of metabolism, behaviour and personality. Phil. Trans. R. Soc. B. 365, 3969-3975.

Houston AI, McNamara JM. 2014 Foraging currencies, metabolism and behavioural routines. J. Anim. Ecol. 83, 30-40.

Hulbert, A. J., Pamplona, R., Buffenstein, R., & Buttemer, W. A. 2007. Life and death: Metabolic rate, membrane composition, and life span of animals. Physiological Reviews, 87(4), 1175–1213.

Kulinčević J, M., Rothenbuhler W, C. 1982 Selection for length of life in the honeybee (*Apis mellifera).* Apidologie 13, 347-352.

Katz K, Naug D. 2020 A mechanistic model of how metabolic rate can interact with resource environment to influence foraging success and lifespan. Ecol. Model. 416, 108899.

Laskowski KL, Moiron M, Niemelä PT, 2021. Integrating behavior in life-history theory: Allocation versus acquisition? Trends Ecol. Evol. 36:132-138.

LaZerte, S. E., Reudink, M. W., Otter, K. A., Kusack, J., Bailey, J. M., Woolverton, A., Paetkau, M., de Jong, A., & Hill, D. J. 2017. feedr and animalnexus.ca: A paired R package and user-friendly Web application for transforming and visualizing animal movement data from static stations. Ecology and Evolution, 7(19), 7884–7896.

Le Galliard J-F, Paquet M, Cisel M, Montes-Poloni L. 2013 Personality and the pace-of-life syndrome: variation and selection on exploration, metabolism and locomotor performances. Funct. Ecol. 27, 136-144.

Margotta, J. W., Roberts, S. P., & Elekonich, M. M. 2018. Effects of flight activity and age on oxidative damage in the honey bee, Apis mellifera. Journal of Experimental Biology, 221(14).

Melvin, R. G., Van Voorhies, W. A. and Ballard, J. W. O. 2007. Working harder to stay alive: metabolic rate increases with age in Drosophila simulans but does not correlate with life span. J. Insect Physiol. 53, 1300-1306.

Mugel, S. G., & Naug, D. 2020. Metabolic rate shapes phenotypic covariance among physiological, behavioral, and life-history traits in honeybees. Behavioral Ecology and Sociobiology, 74(10), 1–9.

Mugel S, Naug D. Metabolic rate diversity influences group-level performance in honeybees. Am. Nat. In press.

Nespolo, R. F. and Franco, M. 2007. Whole-animal metabolic rate is a repeatable trait: a meta-analysis. Journal of Experimental Biology, 210(11), 2000–2005.

Neukirch, A. 1982. Dependence of the life span of the honeybee (Apis mellifica) upon flight performance and energy consumption. Journal of Comparative Physiology 1982 146:1, 146(1), 35–40.

Nielsen D, Page RE, Jr., Crosland MWJ. 1994 Clinal variation and selection of MDH allozymes in honey bee populations. Experientia 50, 867-871.

Niitepõld, K., & Hanski, I. 2013. A long life in the fast lane: Positive association between peak metabolic rate and lifespan in a butterfly. Journal of Experimental Biology, 216(8), 1388–1397.

Okada, K., Pitchers, W. R., Sharma, M. D., Hunt, J., & Hosken, D. J. 2011. Longevity, calling effort, and metabolic rate in two populations of cricket. Behavioral Ecology and Sociobiology, 65(9), 1773–1778.

Oklejewicz, M., & Daan, S. 2002. Enhanced longevity in Tau mutant Syrian hamsters, Mesocricetus auratus. Journal of Biological Rhythms, 17(3), 210–216.

Pearl, R. 1928. The Rate of Living. New York: Knopf.

Perry, C. J., Søvik, E., Myerscough, M. R., & Barron, A. B. 2015. Rapid behavioral maturation accelerates failure of stressed honey bee colonies. Proceedings of the National Academy of Sciences of the United States of America, 112, 3427–3432.

Prado, A., Requier, F., Crauser, D., Le Conte, Y., Bretagnolle, V., & Alaux, C. 2020. Honeybee lifespan: the critical role of pre-foraging stage. Royal Society Open Science, 7(11), 200998.

Pyke GH, Pulliam HR, Charnov EL. 1977 Optimal foraging: A selective review of theory and tests. Q. Rev. Biol. 52, 137-154.

Réale D, Garant D, Humphries MM, Bergeron P, Careau V, Montiglio P-O. 2010 Personality and the emergence of the pace-of-life syndrome concept at the population level. Phil. Trans. R. Soc. B. 365, 4051-4063.

Ricklefs RE, Wikelski M. 2002 The physiology/life-history nexus. Trends Ecol. Evol. 17, 462-468.

Rubner, M. 1908. Das Problem der Lebensdauer und Seine Beziehungen zu Wachstum und Ernährung. Munich: R. Oldenbourg.

Rueppell, O., Bachelier, C., Fondrk, M. K., & Page, R. E. 2007. Regulation of life history determines lifespan of worker honey bees (Apis mellifera L.). Experimental Gerontology, 42(10), 1020–1032.

Salin, K., Auer, S. K., Rey, B., Selman, C., & Metcalfe, N. B. 2015. Variation in the link between oxygen consumption and ATP production, and its relevance for animal performance. Proceedings of the Royal Society B: Biological Sciences, 282(1812).

Schippers, M. P., Dukas, R., & McClelland, G. B. 2010. Lifetime- and caste-specific changes in flight metabolic rate and muscle biochemistry of honeybees, Apis mellifera. Journal of Comparative Phys, 180(1), 45-55

Schmid-Hempel P. 1987 Efficient nectar-collecting by honeybees I. economic models. J. Anim. Ecol. 56, 209-218.

Schmid-Hempel P, Kacelnik A, Houston AI. 1985 Honeybees maximize efficiency by not filling their crop. Behav. Ecol. Sociobiol. 17, 61-66.

Schmid-Hempel P, Wolf T. 1988 Foraging effort and life span of workers in a social insect. J. Anim. Ecol. 57, 509-521.

Seeley, T. D. 1982. Adaptive significance of the age polyethism schedule in honeybee colonies. Behavioral Ecology and Sociobiology 1982 11:4, 11(4), 287–293.

Seeley, Thomas D. The Wisdom of the Hive : the Social Physiology of Honey Bee Colonies. Cambridge, Mass: Harvard University Press, 1995. Print.

Speakman, J. R. 2005. Body size, energy metabolism and lifespan. Journal of Experimental Biology, 208(9), 1717–1730.

Speakman, J. R., & Selman, C. 2011. The free-radical damage theory: Accumulating evidence against a simple link of oxidative stress to ageing and lifespan. BioEssays, 33(4), 255–259.

Speakman, J. R., Talbot, D. A., Selman, C., Snart, S., McLaren, J. S., Redman, P., Krol, E., Jackson, D. M., Johnson, M. S., & Brand, M. D. 2004. Uncoupled and surviving:

individual mice with high metabolism have greater mitochondrial uncoupling and live longer. Aging Cell, 3(3), 87–95.

Speakman, J. R., van Acker, A. and Harper, E. J. 2003. Age-related changes in the metabolism and body composition of three dog breeds and their relationship to life expectancy. Aging Cell 2, 265-275.

Stamps, J. A., & Biro, P. A. 2016. Personality and individual differences in plasticity. Current Opinion in Behavioral Sciences, 12, 18–23.

Stoffel, M. A., Nakagawa, S., & Schielzeth, H. 2017. rptR: repeatability estimation and variance decomposition by generalized linear mixed-effects models. Methods in Ecology and Evolution, 8(11), 1639–1644.

Tenczar, P., Lutz, C. C., Rao, V. D., Goldenfeld, N., & Robinson, G. E. 2014. Automated monitoring reveals extreme interindividual variation and plasticity in honeybee foraging activity levels. Animal Behaviour, 95, 41–48.

Van Voorhies, W. A., & Ward, S. 1999. Genetic and environmental conditions that increase longevity in Caenorhabditis elegans decrease metabolic rate. Proceedings of the National Academy of Sciences of the United States of America, 96(20), 11399–11403.

Visscher PK, Dukas R. 1997 Surivorship of foraging honey bees. Insectes Soc. 44, 1-5.

Winston, M. L. 1994. The biology of the honeybee. B-Beauvechain, Nauwelaerts Ed.

Wolf THJ, Schmid-Hempel P, Ellington CP, Stevenson RD. 1989 Physiological correlates of foraging efforts in honey-bees: oxygen consumption and nectar load. Funct. Ecol. 3, 417-424.

Wolf TJ, Schmid-Hempel P. 1990 On the integration of individual foraging strategies with colony ergonomics in social insects: nectar-collection in honeybees. Behav. Ecol. Sociobiol. 27, 103-111.

Ydenberg RC, Schmid-Hempel P. 1994 Modelling social insect foraging. Trends in Ecology and Evolution 9, 491-493.

Ydenberg RC, Welham CVJ, Schmid-Hempel R, Schmid-Hempel P, Beauchamp G. 1994 Time and energy constraints and the relationships between currencies in foraging theory. Behav. Ecol. 5, 28-34.

APPENDIX A: SUPPLEMENTARY MATERIAL FOR CHAPTER 1

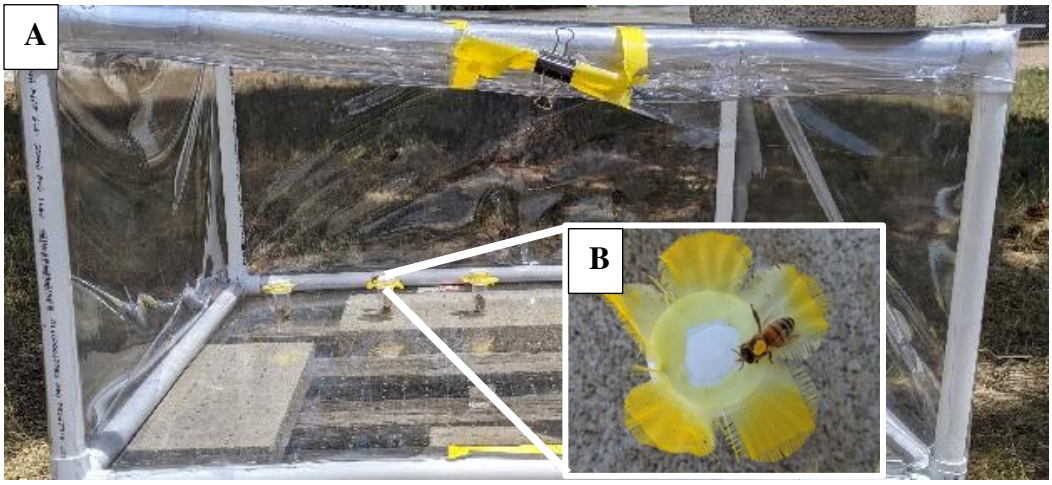
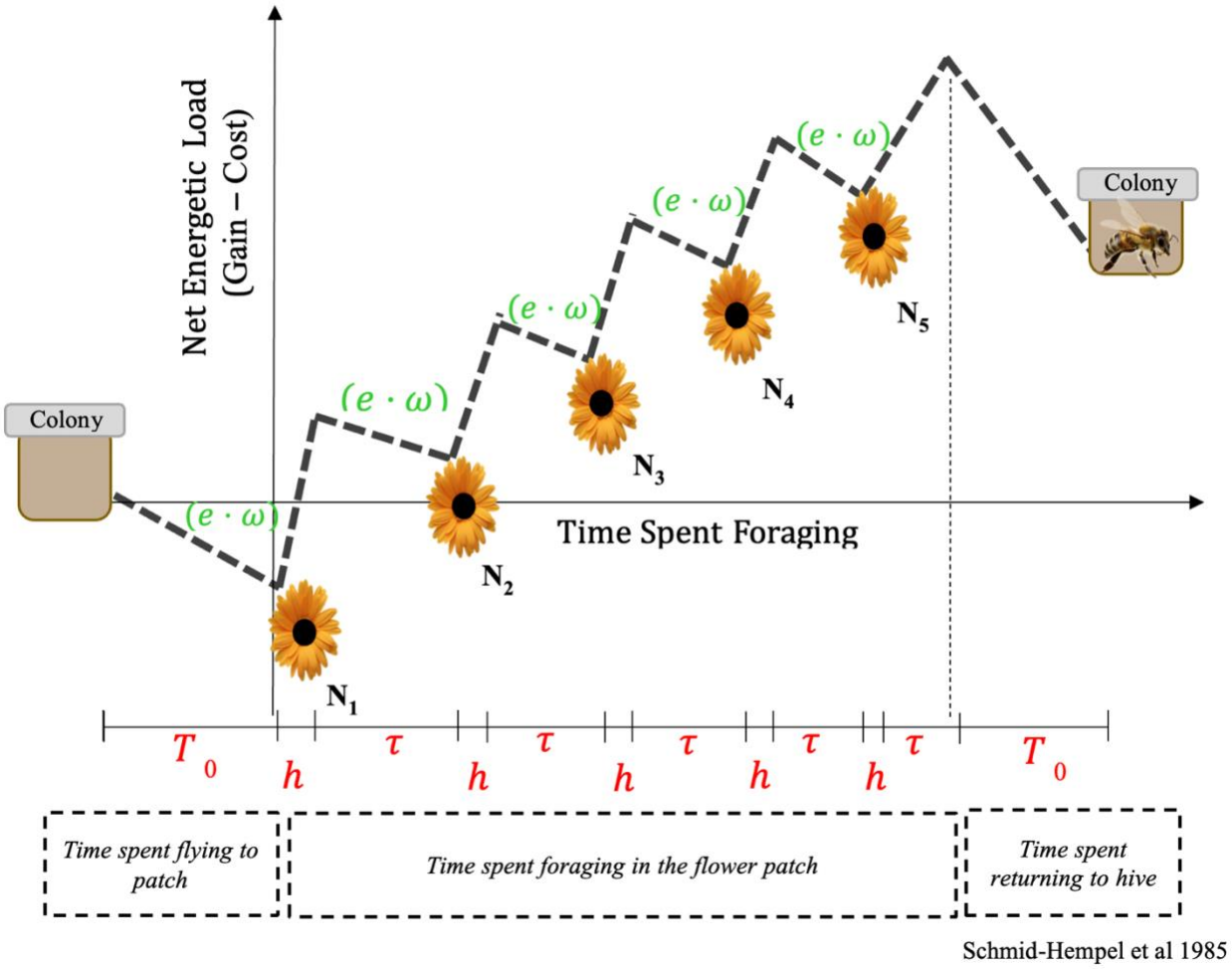


Figure SA.1. The foraging arena (A) with an insert (B) showing a forager drinking from an artificial flower.



Schmid-Hempel et al 1985

Gross Energetic Gain from a foraging trip:

$$G = N \cdot e \cdot \omega \quad (1)$$

Energetic Cost of a foraging trip:

$$C_p = a_0 \cdot (N-1) \cdot \tau + a(\omega + 2\omega + 3\omega + \cdots + [N-1](\omega + r)) \cdot \tau + + a_h \cdot N \cdot h \quad (2a)$$

$$C_p = a_0 \cdot (N-1) \cdot \tau + a \cdot \frac{N \cdot (N-1)}{2} \cdot (\omega + r) \cdot \tau + a_h \cdot N \cdot h \quad (2b)$$

$$C_T = a_0 \cdot \tau + (a_0 + a(N \cdot (\omega + r))) \cdot \tau_0 \quad (3)$$

$$C = C_p + C_T \quad (4)$$

Trip Time of a foraging trip:

$$T = 2 \cdot \tau_0 + (N-1) \cdot \tau + N \cdot h \quad (5)$$

Figure SA.2. Energetics of a single foraging trip shown as the net energetic load as a function of time after a foraging bee leaves the hive, forages in a flower patch, and returns to the hive. Parameters from the foraging model (Schmid Hempel et al. 1985) are overlayed on the diagram.

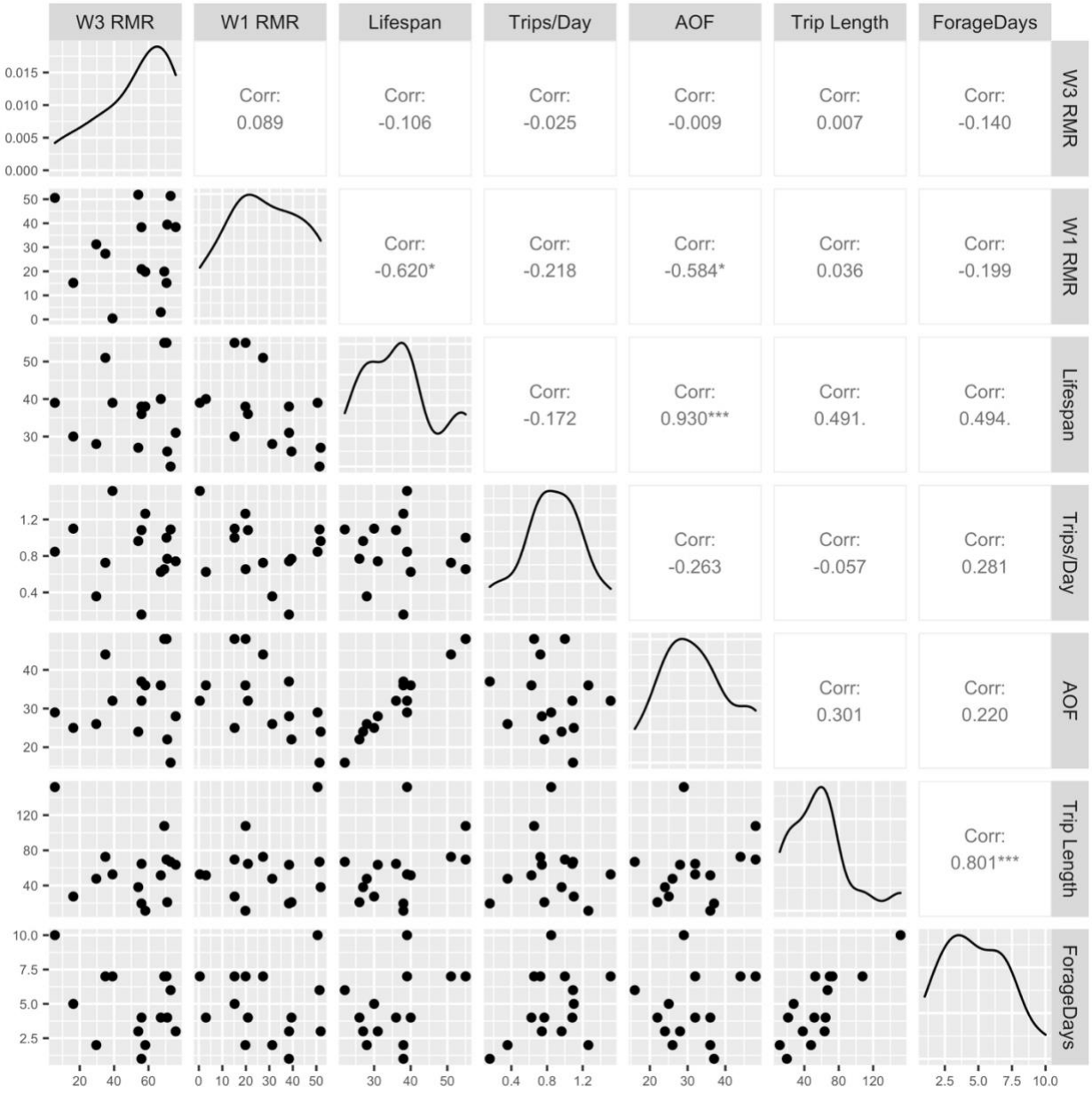


Figure SB.1. Correlation matrix showing Pearson product moment correlation coefficients with significance p values indicated by asterisks. Diagonals show density plots for each continuous variable.

Table SB.1. For each response variable, ANOVA results are displayed for fixed effects in both the full model (with interaction) and reduced model (without interaction) with significance bolded.

	Phenotype		**Birthdate**		**Phenotype*Birthdate**	
Lifespan	$F_{1,83} = 1.01$	$p = 0.32$	$F_{1,83} = 3.74$	$\mathbf{p = 0.002}$	$F_{1,83} = 1.59$	$p = 0.15$
...Reduced Model	$F_{1,83} = 4.57$	$\mathbf{p = 0.03}$	$F_{1,83} = 3.81$	$\mathbf{p = 0.001}$	--	--
AOF	$F_{1,83} = 4.30$	$\mathbf{p = 0.04}$	$F_{1,83} = 14.37$	$\mathbf{p < 0.001}$	$F_{1,83} = 4.31$	$\mathbf{p = 0.04}$
...Reduced Model	$F_{1,83} = 4.95$	$\mathbf{p = 0.02}$	$F_{1,83} = 11.49$	$\mathbf{p = 0.001}$	--	--
Daily Trip Frequency	$F_{1,83} = 0.10$	$p = 0.75$	$F_{1,83} = 5.74$	$\mathbf{p < 0.001}$	$F_{1,83} = 1.83$	$p = 0.09$
...Reduced Model	$F_{1,83} = 0.38$	$p = 0.55$	$F_{1,83} = 4.81$	$\mathbf{p < 0.001}$	--	--
Trip Length	$F_{1,83} = 0.22$	$p = 0.64$	$F_{1,83} = 0.17$	$p = 0.99$	$F_{1,83} = 0.74$	$p = 0.63$
...Reduced Model	$F_{1,83} = 1.76$	$p = 0.19$	$F_{1,83} = 0.64$	$p = 0.71$	--	--