THESIS

EVALUATING METABOLIC RATE, SURVIVAL, FEEDING BEHAVIOR AND ENERGETIC DEMAND IN TWO GENOTYPES OF APIS MELLIFERA

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ABSTRACT

EVALUATING METABOLIC RATE, SURVIVAL, FEEDING BEHAVIOR AND ENERGETIC DEMAND IN TWO GENOTYPES OF APIS MELLIFERA

In animals, metabolic processes play a pivotal role in shaping behavior and physiology, particularly in organisms whose energetic demands are closely linked to their performance in nature. In social insects like honeybees, variation in metabolic rate and energy intake can influence individual performance, leading to colony-level outcomes. However, metabolic processes can be influenced by both genotype and resource availability, but the relative contributions of these factors to individual performance and colony-level outcomes remain unclear.

This study takes advantage of two known genotypes of honeybee (slow, or SS and fast or FF) that can differ in metabolism. Using these metabolic genotypes, I examined how genotype and carbohydrate availability interact to shape physiological performance, short-term survival and feeding behaviors. Physiological performance was evaluated using standard metabolic rate, maximum metabolic rate, and aerobic scope. Workers of the two genotypes were provided either low (20%) or high (40%) sucrose diet treatments in a controlled laboratory environment, and then their metabolic rates were measured. Survival over 24 hours of the two genotypes was tracked under conditions of starvation, and with access to sucrose solutions of a range of concentrations. Feeding behavior of the two genotypes was compared by evaluating gustatory responsiveness to different concentrations of sucrose, as well as by evaluating hunger-driven feeding.

Contrary to expectations based on prior work with these genotypes, metabolic traits did not differ significantly between SS and FF genotypes or between low or high sucrose diet treatment, and no genotype × diet interactions were detected. Short-term survival was strongly influenced by sucrose availability, with bees fed sucrose solutions showing substantially lower mortality risk than unfed bees. In both SS and FF bees, gustatory responsiveness increased with increased sucrose concentration, with FF bees showing slightly, but not significantly, higher responsiveness than SS. The hunger assay showed initially high intake, with similar temporal intake trajectories for both SS and FF bees.

Together, these results suggest that immediate energetic state and dietary treatments influence short-term survival, and hunger driven feeding behavior more than genotype under a controlled laboratory environment. Only gustatory responsiveness differed consistently between the two genotypes, indicating that sensitivity to food rewards can remain genotype dependent even if metabolic traits do not differ significantly. These findings suggest that metabolic genotype effects in honeybees are context dependent. These effects may be expressed more strongly under conditions of sustained energetic demand. This highlights how energetics mold honeybee behavior and performance, and influence overall metabolic demand and survival in them.

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DEDICATION

I am dedicating this thesis to my city, Kolkata—to me, the most beautiful, intellectually vibrant, and deeply reflective place in the world. It has shaped me in ways beyond what I can fully express, often feeling like a guiding force in my upbringing. A city where art, literature, and dialogue are constantly nurtured, has taught me to question, to think critically, and to continually learn and unlearn with openness. May it never stop standing for truth, curiosity, and resilience. You will always be my first love.

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Introduction

Energy is the elementary currency for all physiological processes organisms use during their life. As a result, variation in energy demand and metabolic expenditure can determine how individuals maintain important physiological functions and manage environmental challenges (Cody, 1966; Leyria et al., 2025; Sibly & Calow, 1986). As energy reserves are finite, differences in energetic demand can constrain physiological performance under resource limitation and influence how individuals cope with stress in the environment. In insects, particularly when food is limiting, energetic constraints are often discussed with regards to tradeoffs in energy allocation among growth, reproduction, survival, and performance (Serediuk et al., 2024; Tao et al., 2023; Zera & Harshman, 2001). Directly quantifying life-history tradeoffs can be challenging and hence physiological proxies are often used to measure energetic demand and performance (Audzijonyte & Richards, 2018; Kooijman et al., 2008; Mikkelsen et al., 2023; Sowersby et al., 2019; Thunell et al., 2023).

The metabolic rate of an organism provides one such measurable proxy for energetic demand (Biro & Stamps, 2010; Careau & Garland, 2012; Kim et al., 2022; Shokri et al., 2024). Variation in metabolic rates is often understood using the pace of life syndrome (POLS), which places organisms on a continuum from slow to fast in their pace of life. The pace of life syndrome proposes that metabolism, behavior, and life-history traits are correlated along a slow–fast axis. Taxa or individuals with slow metabolic strategies are predicted to have lower energy demand and activity rates, higher stress tolerance and lifespan, whereas taxa or individuals with fast metabolic strategies are predicted to have higher energy demand and turnover and activity rates traded off against shorter life span (Dammhahn et al., 2018; Promislow and Harvey, 1990;

Réale et al., 2010; Ricklefs and Wikelski, 2002). Multiple studies find support for the pace of life syndrome across a variety of animal taxa (Cassano & Naug, 2022; Godin et al., 2022; Hall et al., 2015; Debecker et al., 2016; Mugel & Naug, 2022)

However, other studies across taxa and levels of biological organizations do not support the pace of life syndrome, with metabolic rate, behavior, and life-history traits often covarying only weakly, particularly at the intra-specific level. For example, individuals within the same population do not always show the expected trade-off between higher metabolic rate and reduced survival but instead are influenced by the environmental conditions they are facing (Royauté et al., 2018a; Van De Walle et al., 2023). Differences in how individuals acquire and allocate energy can often mask the expected trade-offs as individuals with greater access to resources are capable of investing simultaneously in both survival and performance, and this weakens the pace of life syndrome predictions (Smallegange & Guenther, 2025). In addition to this, ecological factors such as variation in mortality risk can also cause alteration in the life-history strategies of organisms, such that organisms experiencing lower mortality can evolve longer lifespans despite high energetic expense. For example, bats use large amounts of energy for flight, but they often have longer lifespan than expected with respect to their body size because flying helps them avoid predators and hence, they can invest more in somatic maintenance and survival breaking the expected link between high metabolism and short lifespan (Wilkinson & South, 2002). Together, these findings suggest that while the pace of life syndrome provides a strong general framework, its predictions may be modified or obscured by ecological context.

One such ecological context that might influence the relationships between metabolic and behavioral traits, and survival is resource availability (Arnold et al., 2021; Burton et al., 2011; Niemelä & Dingemanse, 2018; Royauté et al., 2018; Strijker et al., 2023). To understand if the

pace of life syndrome describes organismal traits, and whether that depends upon resource availability, it can be useful to measure the baseline energetic costs required for maintenance, termed standard metabolic rate (SMR), and the energy used during intense activity, termed maximum metabolic rate (MMR). The difference between maximum metabolic rate and standard metabolic rate is defined as aerobic scope (AS) of an organism. Aerobic scope represents the energetic capacity available for activities beyond maintenance like growth, physiological activities, etc. (Auer et al., 2017, 2016a; Fry, 1971; Levet et al., 2024).

Variation in metabolic rate is especially important in social insects, where individual metabolism can influence division of labor, and scale up to colony-level outcomes (Lemanski et al., 2021; Naug, 2024). In honeybees, metabolic rate can be influenced by a genetic polymorphism affecting the enzyme malate dehydrogenase-1 (MDH-1). Individuals with the fast allele (FF) typically have higher metabolic rates, whereas individuals with the slow allele (SS) typically have lower metabolic rates (I. R. Coelho & Mitton, 1988; Mugel & Naug, 2022). Such metabolic differences at the individual level can impact task specialization. For example, workers with higher metabolic rates are expected to perform energetically demanding activities like sustained flights, while lower-metabolic rate individuals are expected to conserve energy and tolerate stress more efficiently (Dornhaus et al., 2012; Harrison & Fewell, 2002; Naug, 2024; Rüppell et al., 2004). However, bees with intrinsically high metabolic rates can only perform at their peak if sufficient food is available. Similarly, bees with intrinsically low metabolic rates may not benefit as much from high food availability but may be able to maintain performance even if food is limited (Cassano & Naug, 2022; Reade et al., 2019).

Food availability to bees varies widely in nature, as sugar concentration in nectar varies by plant species and environment (Carvalheiro et al., 2014; Lun et al., 2025; Pacini et al., 2003).

Likewise, climate change and land-use changes can alter flowering phenology and nectar production, impacting the spatio-temporal availability of carbohydrate resources for pollinators (Carvell et al., 2006; Kortsch et al., 2024; Schmidt et al., 2023). Differences in both food availability and intrinsic metabolism are likely to affect foraging decisions and behavior of individual bees (Cassano & Naug, 2022). Thus, to better understand how individuals with different inherent metabolic rates balance energetic demands and performance, it is crucial to measure their physiology and behavior across different levels of resource availability. This can be done by manipulating carbohydrate availability which strongly impacts cognition, lifespan, and metabolic performance in bees to modify energetic intake (Bouchebti et al., 2022; Braglia et al., 2024; Cassano & Naug, 2022; Mugel & Naug, 2022; Najarpoor et al., 2025). This provides a controlled and ecologically relevant experimental approach for measuring how energy availability shapes metabolic rate, survival, and behavior.

Honeybee genotypes (FF and SS) that differ in metabolic traits have previously found to be associated with differences in physiology and behavior (Cassano & Naug, 2022; Mugel & Naug, 2022). But it remains unclear whether these differences are consistently expressed across all the physiological and behavioral traits and how strongly they depend on ecological conditions such as resource availability. As the quality and quantity of nectar vary widely in the nature (Corbet et al., 1979; Heil, 2011; Herrera et al., 2006), it is important to understand how intrinsic metabolic genotypes and carbohydrate availability together influence metabolic rate, feeding behavior, and survival in honeybees.

To address this, I examined two *Apis mellifera* genotypes that differ in metabolic traits (SS: slow; FF: fast) to evaluate (1) how they differ in standard metabolic rate, maximum metabolic rate, aerobic scope when maintained under low (20%) and high (40%) sucrose diets in

laboratory conditions; (2) whether short-term survival differed when bees were provided different total amounts of 30% sucrose solution (0, 10, 30 μL, or ad libitum; (3) whether they differ in gustatory responsiveness across a gradient of sucrose concentration; and (4) whether they differ in hunger-driven feeding behavior.

Methods

Study System

The western honeybee (*Apis mellifera*) is a eusocial insect with haplodiploid sex determination. Female worker bees perform tasks such as brood care, colony maintenance and foraging through division of labor in a synchronized way to maintain the colony (Lattorff & Moritz, 2013; Robinson, 1992). These tasks differ with respect to energy demands. For example, foragers experience the highest energetic demand in the colony due to sustained flight and food collection, this life-stage is specifically relevant for examining interaction between intrinsic metabolic genotype and variation in energetic conditions in the environment (Cassano & Naug, 2022) . Additionally, as noted above, honeybees possess a natural genetic polymorphism at the malate dehydrogenase-1 (MDH-1) locus, which enables tests of the pace of life syndrome mechanistically under controlled laboratory conditions. Due to their immense economic importance to agriculture(Khalifa et al., 2021) the species is an established model organism. Thus, honeybees serve as an ideal system for investigating the link between metabolic genotypes and performance, behavior, and survival (Cassano & Naug, 2022; Johnson, 2010; Perez & Johnson, 2025; Scheiner et al., 2021), particularly regarding how genotype affects group-level outcomes (Mugel & Naug, 2022; Naug & Tait, 2021).

Rearing SS and FF bees for experiments

To address my objectives, I used SS and FF genetic lines of honeybees (*Apis mellifera)*, which differ in malate dehydrogenase (MDH-1) allotypes where the S allele is associated with lower metabolic rate and the F allele with higher metabolic rate (Coelho & Mitton, 1988; Harrison & Fewell 2002). Male drones of known MDH-1 genotypes were used for the artificial insemination of unrelated virgin queens of matching MDH-1 genotypes to create the following two crosses: S x SS and F x FF producing slow (SS) and fast (FF) offspring, respectively. Three FF colonies and five SS colonies (hereafter, "source colonies") were established using female worker bees from starter colonies and a queen of the corresponding genotype (refer to Mugel & Naug, 2022 for further details). Brood frames were pulled from these source colonies when the pupae were almost ready to emerge and incubated at 32ºC and 60% relative humidity (RH) overnight. Newly emerged bees from SS and FF source colonies were color-marked on the abdomen and thorax using non-toxic marker paint to indicate genotype, source colony, and date of emergence. For metabolic rate experiments, one-day-old, marked bees were directly assigned to cages maintained under controlled conditions with either 20% or 40% sucrose diets treatments, and allowed to mature for a minimum of 15 and maximum of 20 days. Sucrose was provided with a feeder syringe. Additionally, a cotton ball soaked in the same sucrose concentration as the feeder syringe was placed externally over the holes of the cage and replaced routinely to ensure uninterrupted access to the respective sucrose concentration in case the feeder malfunctioned temporarily (via formation of an air bubble in the capillary) and to prevent cage desiccation. A small amount of bee bread (a nutrient-rich mixture of pollen, nectar, and honey that bees mix with their digestive enzymes) that was pre-collected and stored in laboratory refrigerator was affixed with the honeycomb to support nutritional needs of newly emerged and young bees from 1-9 days old (Yang et al., 2021). After 9 days, the bees only had access to the

assigned sucrose solution. Bees were maintained on their respective diets for 15-20 days at 27ºC and 65-70% Relative Humidity before measuring metabolic rates. Approximately 32 bees of one genotype were maintained per cage, and SS and FF bees were kept separately by diet treatment for a total of four types of cages.

Similarly, for survival and gustatory responsiveness and hunger assays one-day old, marked bees were held in a foster colony to provide a common environment while they matured prior to experimentation. Bees kept in a foster colony were collected when they reached the appropriate age for the survival experiment, and for gustatory responsiveness and hunger assays (Figure 1). For all my experiments, worker bees of age between 15–28 days were used, as this age window corresponds to typical foraging age of honeybees (Vance et al., 2009).

Experimental Approaches

Measurement of Metabolic Rates and Calculation of Aerobic Scope

Each bee collected for measurement of metabolic rate was first weighed, harnessed in a plastic straw using a small wire, and satiated with their assigned concentration of sucrose. Bees were then kept inside the incubator overnight (13-16.5hrs) to ensure a post-absorptive state before measuring first standard metabolic rate, then maximum metabolic rate.

Standard metabolic rate was measured using a flow-through carbon-dioxide respirometry with a Fox Box setup (Sable Systems). Each harnessed bee was placed inside a 50 mL respirometry chamber and ambient air, scrubbed of H_2O and CO_2, was supplied to the chamber at a constant flowrate of 250 mL/min. The CO_2 concentration in the excurrent airflow was recorded for 10 minutes continuously and was used together with the known flow rate, to calculate CO_2 production rate ($\dot{V}CO_2$) for each bee. A baseline measurement (blank run) was done prior to each measurement using an empty identical chamber for 1 minute to calculate corrected CO_2 output

for each bee. The continuous 2-minute window with the lowest variance in CO_2 production was used to calculate standard metabolic rate. After running a batch of bees, they were fed ad libitum with their respective diet treatment (sucrose concentration) and kept inside an incubator for 30-90 minutes before measuring maximum metabolic rate.

To determine maximum metabolic rate, CO_2 output was measured by placing each bee in a clear 250 mL sealed glass chamber and ambient air was passed through the chamber at a constant flow rate of 750mL/min for 10 minutes. Before each sample run, a blank run was performed for 1 minute. Flight (wing beating) was monitored constantly, and phases of active flight were marked. Flight was stimulated by agitating the bee by gently and continuously shaking the chamber and using a light source above the chamber. Maximum metabolic rate was calculated from the 60-second window with lowest variance of sustained wing beating (rolling flight) identified from the CO_2 production during the active flight period. The values for both standard metabolic rate and maximum metabolic rate were converted to a power output (milliwatt) by multiplying it by 21.4 J m L^{-1} and dividing by 3600 J hr^{-1} then weight corrected (mW/g) (Cassano & Naug, 2022; Feuerbacher et al., 2003; Rylance, E. 2025; Mugel & Naug, 2022.). Thus, although standard and maximum metabolic rate was calculated using different lengths of time (two minutes and one minute, respectively), both were standardized to comparable metabolic rates prior to analysis.

Aerobic scope (AS) was calculated by subtracting standard metabolic rate from maximum metabolic rate for each bee (AS=MMR-SMR).

Survival Analysis

Bees were collected, strapped, and satiated with 30% sucrose and kept at 27ºC and 65-70% relative humidity for 6 hours (equalization step) prior to experimentation. Following

equalization, bees were fed one of four treatment volumes of 30% sucrose solution (0 µL, 10 µL, 30 µL, or ad libitum) only once right at the beginning of the experiment. The sucrose solution was the only source of water, so the 0µl treatment received neither sugar nor water. Their survival was monitored every 6 hours for 24 hours i.e., a total of 5 time points (0, 6,12,18 and 24 hours) (Mayack & Naug, 2009).

Gustatory Responsiveness

Bees were collected, strapped, and satiated with 30% sucrose and kept at 27ºC and 65-70% relative humidity for 6 hours (equalization step) prior to experimentation. The antenna of the strapped bees was touched with a drop of sucrose and whether they extended their proboscis or not was recorded by using binary system (i.e., 1 for proboscis extension and 0 for no extension). Six sucrose concentrations in the sequence 0.1%, 0.3%,1%, 3%, 10% and 30% were used to test gustatory responsiveness in each bee (Bitterman et al., 1983; Mayack & Naug, 2009). All the bees in a test batch were stimulated with the same sucrose concentration before moving to the next concentration and between two concentrations the antennae of each bee were touched with water drops.

Hunger Assay

The same batch of bees used in the gustatory responsiveness assay described above was also used for the hunger assay. Immediately after completing the gustatory responsiveness assay, the same batch of bees were provided ad libitum 30% sucrose and the hunger assay began following the protocol of Mayack & Naug, (2009). Bees were fed with 30% sucrose solution every 6 hours for 24 hours and the amount of sucrose intake was recorded at each time point (0^{th}, 6^{th}, 12^{th},18^{th} and 24^{th} respectively). The bees were kept in the same incubator environment for the whole period.

Statistical Analyses

Data analysis was done in R (v4.5.1) (R Core Team, 2026) and separate statistical models were fitted for each experiment.

Metabolic Trait Analyses

Metabolic rates (SMR, MMR and AS) were analyzed using linear mixed models with two different genotypes and two sucrose treatments and their interaction as fixed effects with source colony included as a random intercept. To meet assumptions of normality and homoscedasticity of residuals, metabolic rates were log transformed for analysis. Models were fitted using the *lme4* package (Bates et al., 2015), with p-values obtained using *lmerTest* (Kuznetsova et al., 2017). ANOVA III with Satterthwaite's approximation, was used to obtain appropriate tests of fixed effects in mixed models with unbalanced designs and find degrees of freedom. Estimated marginal means were calculated using *emmeans* (Lenth, 2023), and I estimated treatment means while controlling for multiple comparisons with Tukey adjustments.

Survival analyses

Kaplan-Meier curves with log-rank test were used to compare survival distributions across genotypes and sucrose treatments in the 24-h survival assay. A log-rank test was used to evaluate whether genotypes differed within each sucrose dose. A Cox proportional hazard model, stratified by source colony, was used to quantify mortality risk where genotype, sucrose dose, and their interaction were treated as fixed effects; Schoenfeld residuals were used to confirm assumption of Cox model (proportional hazard). Survival analyses were conducted using the *survival* package (Therneau, 2020) and Kaplan–Meier plots were generated using *survminer* (Kassambara et al., 2021). Additionally, survival probability (alive or dead at 24 hour) was analyzed using a binomial generalized linear mixed model with a logit link fitted using the *lme4*

package (Bates et al., 2015) where genotype, sucrose dose and their interaction were treated as fixed effects and source colony was the random intercept. Estimated marginal means were obtained using *emmeans* (Lenth, 2023).

Gustatory responsiveness analysis

Binomial GLMM with logit link was employed to analyze the gustatory responsiveness data, where response (proboscis extension of the bees) was evaluated as a function of genotype, sucrose concentration, and interaction. The model was fitted using the *lme4* package (Bates et al., 2015). Bee ID and source colony were treated as random intercepts to account for repeated measures for each bee and colony-level variation, respectively. Type III Wald chi-square tests were performed using the *car* package (Fox & Weisberg, 2019) to evaluate the fixed effects. Estimated marginal means were obtained using *emmeans* (Lenth, 2023) on the logit scale and back-transformed for interpretation. At each sucrose concentration, genotype contrast was evaluated using Sidak-adjusted pairwise test.

Hunger analysis

Consumption of sucrose during the 24-hour hunger assay, both cumulative and for each time interval were analyzed using a linear mixed-effects model (LMM) fitted using the *lme4* package (Bates et al., 2015). Cumulative intake (running total consumption over time) and interval intake (consumption within each time block) were modeled separately to distinguish overall intake from temporal feeding dynamics. In both models, intake was modeled as a function of genotype, time, and their interaction. Bee ID was included as a random intercept to account for repeated measurements on the same individual (individual level variation across time), and source colony was included as an additional random intercept. Type III ANOVA was

performed using the *car* package (Fox & Weisberg, 2019) to evaluate the significance of fixed effects.

Results

Metabolic Rate

Contrary to expectations and other experiments showing differences in metabolism in SS and FF bees (Cassano & Naug, 2022b; Mugel & Naug, 2022), the results from this study showed that standard metabolic rate, maximum metabolic rate, and aerobic scope did not differ significantly by genotype. Additionally, sucrose treatment and the interaction between genotype and sucrose treatment were also not significant. (SMR: genotype $F_{1,1}.62 = 0.33$, $p = 0.63$; sucrose treatment $F_{1,60}.58 = 1.63$, $p = 0.21$; interaction $F_{1,60}.58 = 0.04$, $p = 0.84$; MMR: all p values > 0.44 and AS: all p values >0.38; Fig. 2-4). SS and FF bees showed similar metabolic rates across under both low carbohydrate and high carbohydrate diet.

Survival

Across sucrose doses, SS bees showed slightly higher proportion survival over 24 hours (0.657) than the FF (0.575) bees, but this difference was not statistically significant (log-rank tests, all p values >0.11; Fig. 5a). Consistent with trends in the Kaplan-Meier results, the Cox Model showed no significant main genotype effect (hazard ratio for SS<1, but confidence intervals overlapped). In contrast, mortality risk decreased significantly with increasing sucrose dose, with bees provided higher sucrose volumes (10 µl, 30 µl, and ad libitum) exhibiting reduced hazard relative to the lowest dose, indicating a dose-dependent effect of carbohydrate availability (all p values <0.02). Survival probability at 24 h was significantly affected by sucrose availability, increasing with higher sucrose concentrations (dose effects: 10%: $z = 3.22$, p

= 0.001; 30%: z = 2.23, p = 0.026; ad libitum: z = 2.62, p = 0.0087; Fig. 5b). There was no significant effect of genotype (z = 1.48, p = 0.138), and no genotype × sucrose interaction was detected (all p > 0.09). Together, these results indicate that short-term survival is primarily driven by immediate energetic input, with increasing sucrose availability progressively reducing mortality risk across treatments.

Gustatory Responsiveness

Gustatory responsiveness varied significantly across sucrose concentrations (binomial GLMM; sucrose main effect: $\chi^2_5 = 24.36$, $p < 0.001$; Fig. 6). Genotype had a significant effect on gustatory responsiveness ($\chi^2_1 = 4.53$, $p = 0.033$), with FF bees exhibiting overall higher gustatory responsiveness than SS bees. However, the genotype × sucrose interaction was not statistically significant ($\chi^2_5 = 6.12$, $p = 0.29$), suggesting that the pattern of responsiveness across sucrose concentrations was similar for both SS and FF bees. Sidak-adjusted pairwise comparisons indicated significant differences between genotypes only at 0.1% and 10% sucrose concentrations, respectively.

Hunger Assay

As expected, cumulative sucrose consumption increased over the time in the 24-hour hunger assay (main effect of time: $\chi^2 = 221.25$, df = 4, $p < 0.001$; Fig. 7a) showing the progressive accumulation of intake during the experiment. However, there was no significant difference in cumulative intake between SS and FF bees (genotype effect: $\chi^2 = 0.31$, df = 1, $p = 0.578$), and no genotype × time interaction was detected ($\chi^2 = 2.82$, df = 4, $p = 0.589$). Model-

estimated means suggested that both SS and FF bees exhibited similar trajectories of cumulative sucrose intake across all five time points.

Interval sucrose intake analysis showed a significant effect of time ($\chi^2 = 49.33$, df = 4, $p < 0.001$; Fig. 7b), but neither genotype ($\chi^2 = 1.30$, $p = 0.255$) nor the genotype × time interaction ($\chi^2 = 6.58$, $p = 0.160$) was statistically significant. Both SS and FF bees exhibited a similar temporal pattern, with intake initially high, then declining and increasing again slightly during later intervals.

Discussion

This study investigated if genetically distinct metabolic lines of *Apis mellifera* (SS vs FF) differ in metabolic rate, short-term survival and feeding behavior in controlled laboratory experiments in which carbohydrate availability was manipulated. Contrary to expectations based on previous studies (Cassano & Naug, 2022; Mugel & Naug, 2022), we did not observe uniform differences between SS and FF bees across experiments. Instead, they were limited and context dependent. Metabolic traits (standard metabolic rate, maximum metabolic rate, and aerobic scope) did not differ significantly between genotypes under either low or high sucrose diet treatments, and no significant genotype × diet interactions were observed, suggesting broadly similar physiological performance of SS and FF bees under controlled laboratory conditions used here, with considerable variation among individuals within these groups. In contrast, survival depended strongly on the availability of sucrose solution: bees receiving sucrose solution (10 µl, 30 µl, or ad libitum) showed significantly reduced mortality relative to the unfed (0 µl) bees. While this dose-dependent effect of sucrose availability was expected, a consistent (non-significant) trend of higher survival in SS bees compared to FF bees was observed across treatments. This effect was broadly consistent across genotypes, suggesting that differences in

survival are not driven by how many more unfed bees die, but rather by subtle genotype-linked variation in survival proportion under energetic stress. Such a directional trend, although not statistically significant, is consistent with expectations from the pace of life syndrome, where slower metabolic types prioritize survival over energy-intensive activities (Promislow & Harvey, 1990; Réale et al., 2010; Ricklefs & Wikelski, 2002). Behavioral assays further highlighted experiment-specific effects of sucrose availability. Gustatory responsiveness increased with increasing sucrose concentration, and significantly higher responsiveness was detected in FF bees overall than SS bees. In the hunger assay where sucrose availability was not manipulated, cumulative sucrose consumption elevated predictably over time in both genotypes. Both SS and FF bees exhibited similar temporal intake trajectories characterized by an early decline followed by late recovery, suggesting conserved feeding dynamics under prolonged food deprivation. Taken together, these results indicate that immediate energetic input and dietary context exert a stronger influence on short-term survival and feeding behavior than intrinsic genotypic differences. Importantly, the trend of higher survival, and the finding of significantly lower gustatory responsiveness in SS bees than FF bees both align with predictions from the pace of life syndrome (Careau et al., 2003; Ricklefs & Wikelski, 2002; Scheiner et al., 2004; Wang et al., 2012). However, the absence of corresponding genotype differences in metabolic traits and cumulative sucrose consumption suggests that support for the pace of life syndrome is context dependent and not uniformly expressed under the controlled laboratory conditions used here.

The absence of differences in metabolic traits between SS and FF bees seen here contrasts with the results from earlier work (Cassano & Naug, 2022; Mugel & Naug, 2022) and can likely be explained by three main differences in experimental design: (i) the behavioral and physiological condition the bees at the time of measuring metabolic rates, (ii) the extent of

contrast in carbohydrate concentration imposed in diet, and (iii) criteria for including experimental units.

First, Cassano & Naug (2022) quantified metabolic rates in active foragers exiting the hive, which are individuals performing energetically demanding tasks like sustained flight, whereas in the present study I quantified metabolic rates of bees that were maintained in cages in the laboratory. The bees I used are thus likely to include a broader range of physiological variation than would remain after task specialization or behavioral and physiological filtering as foragers. As foraging is a behaviorally and physiologically selective task typically performed by workers with higher activity levels and activation of specific genetic pathways, the forager population in a social insect colony may represent a filtered subset of individuals in which metabolic differences at genetic levels are more likely to be expressed (Chan et al., 2011; Ingram et al., 2011).

Second, differences in how sucrose treatments were structured across studies may influence the expression of genotypic differences in metabolic rates. Mugel & Naug (2022) imposed a stronger contrast in carbohydrate concentrations, using 15% as low resource and 30% as high resource sucrose diets. Cassano & Naug, (2022) made a contrast in resource environments using 30% and 50% sucrose concentrations that represent the natural range of nectar carbohydrate concentrations collected by foraging honeybees (Seeley, 1986). In my study, I used 20% and 40% sucrose diets, both of which fall under nutritionally adequate and optimal range, respectively, for honeybees (Nicolson, 2022). Though the numerical contrast between high and low sucrose treatments in my study (20%) is comparable to or greater than that in the prior studies, differences in experimental context—including colony foragers versus caged bees, grouped measurements versus individual measurements, and variation in physiological state of

the bees—may determine whether genotype-related metabolic differences are detectable. Hence, the energetic conditions experienced by bees in this experiment may not have imposed a sufficiently strong ecological contrast for genotype-related differences in metabolic demand to emerge under the controlled cage conditions used here.

Third, differences in the level at which metabolic rates were quantified may also lead to differences in results from the prior study. Mugel & Naug (2022) quantified metabolic rates at the group level and did not incorporate the cages in their respirometry experiment that had high mortality. This may have decreased physiological variance among their experimental units. In the present study, I measured metabolic rates at the individual level and retained all the cages irrespective of mortality. As a result, the present dataset likely consists of greater inter-individual physiological variation than reported by Mugel & Naug (2022). Increased physiological variance is known to obscure relationship between physiological state and performance, decreasing statistical power for detecting subtle effects or differences (Rgen Tautz et al., 2003).

Together, these differences in experimental design may explain differences in outcomes and suggest that genotypic differences in metabolic rates are context dependent. This further indicates that predictions from the pace of life syndrome may not be consistently expressed under controlled laboratory conditions and instead emerge under specific conditions like sustained high energetic demand, task specialization and during group level interactions with lower inter-individual physiological variance.

The results of the 24-hours survival assay in our study did not show significant genotypic differences between SS and FF bees across sucrose doses. However, SS bees showed a slightly higher survival overall and a correspondingly lower hazard of mortality as per the Cox model than the FF bees. Although this trend was not statistically significant, the directional trend is

consistent with expectations from the pace of life syndrome. Under the pace of life syndrome, individuals with lower metabolic rate are hypothesized to conserve energy and exhibit increased survival, whereas individuals with higher metabolic rate are hypothesized to incur high energetic costs and exhibit reduced survival, particularly under energetically stressful condition (Biro, 2024; Careau et al., 2003, 2010). However, in the present study, survival over the short term was primarily driven by sucrose rather than genotypic differences between SS and FF bees. It is likely that subtle differences in survival, if they exist, are not easily detected in a short-term experiment like this but may become apparent over longer-term experiments.

Responsiveness to sucrose was the only measure where SS and FF differed consistently. In honeybees, protrusion of the proboscis is used to assess gustatory responsiveness across a range of sucrose concentrations and variation between genotypes or groups is interpreted with respect to specific thresholds of sucrose responsiveness defined as the lowest sucrose concentration that elicits a sensory response (Han et al., 2021; Loney et al., 2012; Scheiner et al., 2004). Because of this variation, genotype differences may only be detected at specific concentration points along the sucrose gradient where response thresholds diverge among individuals. At very low concentrations only highly sensitive individuals respond, whereas at high concentrations most individuals respond, making genotype differences difficult to detect. Although I did not detect a significant genotype by sucrose concentration interaction with our sample sizes, the results suggest that FF bees may have a lower response threshold than SS bees, as they responded strongly to the lowest concentration, while SS bees did not respond strongly until the highest concentration. The concentration-specific differences observed in the gustatory responsiveness results therefore likely indicate variation in sucrose response thresholds of

individuals rather than a uniform genotype effect across the entire sucrose concentration gradient (Page et al., 1998; Scheiner et al., 2004).

In contrast to the results of the gustatory responsiveness assay, the hunger assay did not show any significant genotype difference in cumulative sucrose consumption between SS and FF bees. Instead, both genotypes showed similar sucrose intake patterns across time points, characterized with high initial intake that then declined, followed by a somewhat higher intake at later time points. Genotype differences were expected because genetic polymorphism associated with the MDH-1 locus in honeybees is known to be linked with variation in energetic demand and behavioral traits (Harrison & Fewell, 2002.; Mugel & Naug, 2022). This could potentially influence feeding motivation or gustatory responsiveness and sucrose consumption. These findings indicate that hunger driven feeding behavior was primarily regulated by short-term internal energetic state of the bees rather than the genetic differences in sucrose sensitivity. Previous studies have shown that sucrose responsiveness and hunger in honeybees are plastic traits and can change with feeding history, immediate nutritional state, and foraging experience rather than being fixed characteristics (Page et al., 1998.; Scheiner et al., 2004). Taken together, results of this study suggest a dissociation between sucrose sensory responsiveness and hunger-driven feeding behavior where genetic variation may only be expressed at certain gustatory sensitivity thresholds. This highlights that feeding behavior in honeybees is shaped by interactions between intrinsic metabolic genotypes and short-term energetic state, indicating that hunger driven feeding behavior cannot be predicted from genotypic difference alone but depends on environmental context as well.

Conclusion and Future Research Directions

Across metabolic rates and aerobic scope, SS and FF bees did not show significant differences under the tested sucrose dietary treatments. Similarly, results from the short-term hunger assay and survival assay did not differ between the genotypes. However, clear genotype differences were observed in the gustatory responsiveness assay. The patterns observed suggest that the fast genotype has a lower sensory threshold than the slow genotype.

Based on the patterns of results observed in this study, multiple directions for future research emerge. First, future research could test honeybee physiological and behavioral responses across a broader range of nutritional environments to better understand how extreme energetic limitations in the environment shapes genotype-dependent variation in metabolic performance. Second, in this study I conducted short-term behavioral assays which may limit the ability to detect genotypic differences. Extending experiments over longer time may show effects that may not be evident within this short-term window. Third, while I focused exclusively on carbohydrate availability, protein and lipids are also important nutrients for honeybees, and manipulating different macronutrients together may provide additional insight into how diet interacts with genotypes. Finally, I conducted all the experiments at individual level and extending these experiments to colony-level could help determine if individual level genotypic differences in gustatory responsiveness scale up to influence collective performance in bee colonies.

Overall, this study shows that immediate energetic state can drive short-term physiological and behavioral responses in honeybees more strongly than intrinsic metabolic genotype under controlled laboratory conditions. These results suggest that expected pace-of-life trade-offs may be weakened or obscured when individuals differ in resource acquisition and immediate energetic state, and when environmental conditions shape how energy is allocated

between survival and performance. Such context-dependent effects can prevent consistent links between intrinsic metabolic genotypes and survival or behavioral responses, particularly at the individual level and over short-term experimental periods. Ecologically, these results highlight the importance of energetic variability in the environment in regulating the expression of intrinsic genotypes in honeybees.

Figures

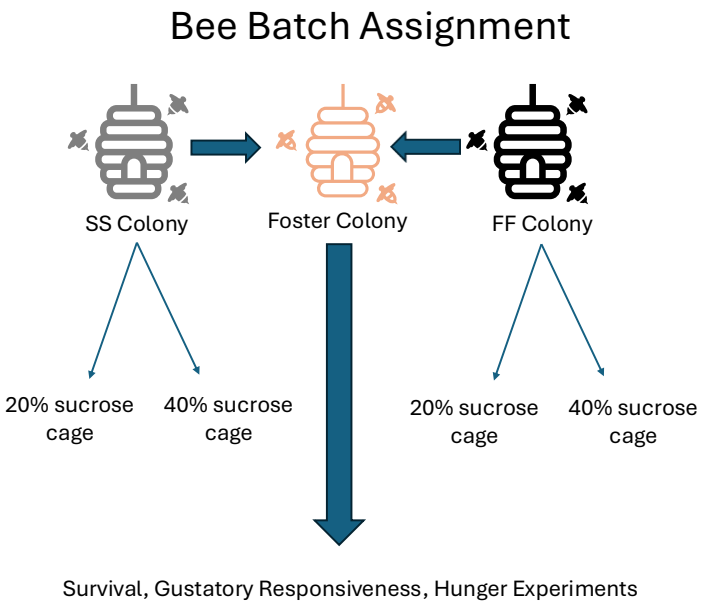

Figure 1. Assignment of honeybee workers to different experiments: Newly emerged workers from SS and FF source colonies were placed either directly into cages receiving either 20 % or 40 % sucrose diets to later measure metabolic rates, or into a foster colony to measure survival, gustatory responsiveness, and hunger.

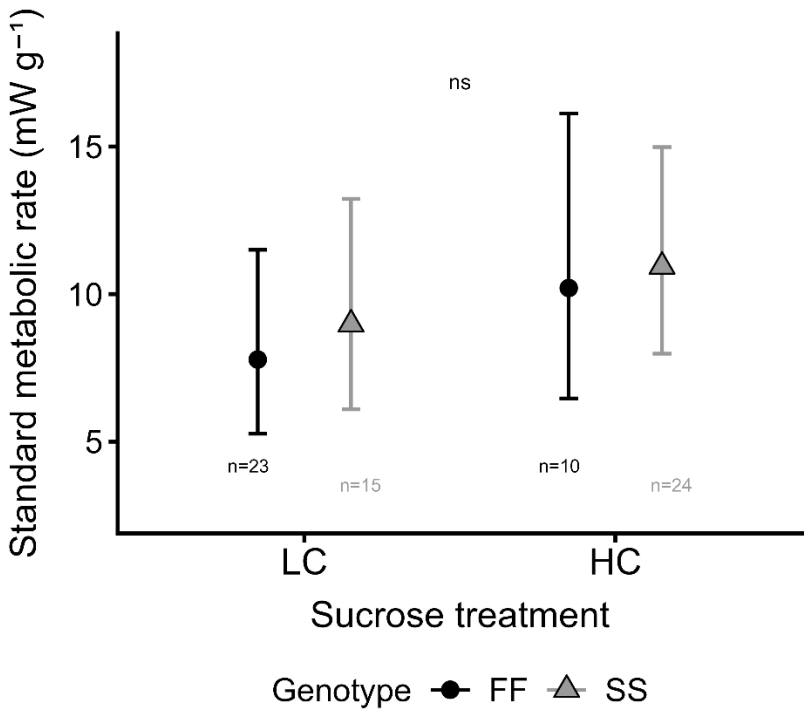

Figure 2. Standard metabolic rates**:** Back-transformed estimated marginal means of standard metabolic rate (mW g^{-1}) for fast (FF) and slow (SS) genotypes under low-carbohydrate (LC, 20%) and high-carbohydrate (HC, 40%) sucrose diets. Values are from linear mixed-effects models fitted on log-transformed data. Error bars represent 95% confidence intervals, back-transformed to the original scale, resulting in asymmetric intervals. Sample sizes (n) are shown below each group.

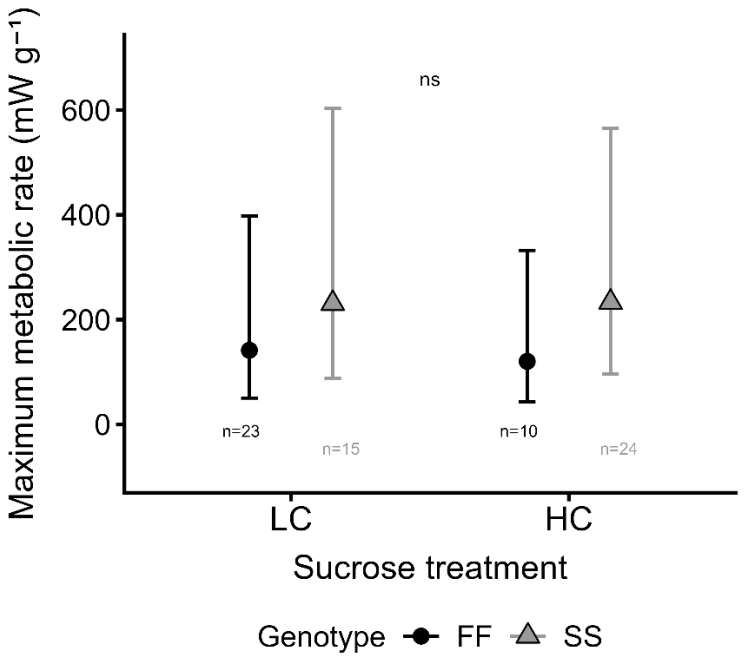

Figure 3. Maximum metabolic rates: Back-transformed estimated marginal means of maximum metabolic rate (mW g^{-1}) for FF and SS bees across sucrose treatments. Error bars indicate 95% confidence intervals derived from log-scale models and back-transformed for graphing. No significant effects of genotype, sucrose treatment, or their interaction were detected.

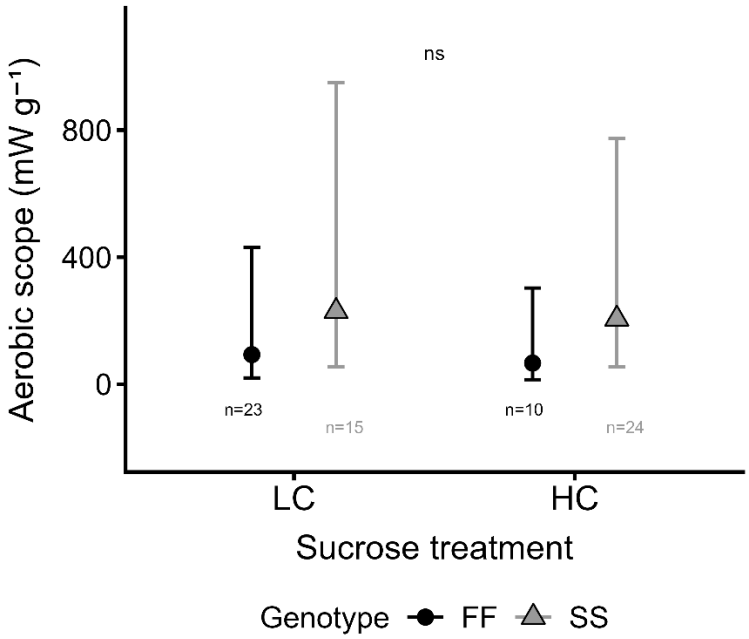

Figure 4. Aerobic scope (MMR − SMR): Back-transformed estimated marginal means of aerobic scope (mW g^{-1}), calculated as the difference between MMR and SMR, across genotypes and sucrose treatments. Aerobic scope represents the metabolic capacity available for activities beyond basic maintenance. Error bars represent 95% confidence intervals on the original scale. Genotype, sucrose treatment, and their interaction were not statistically significant.

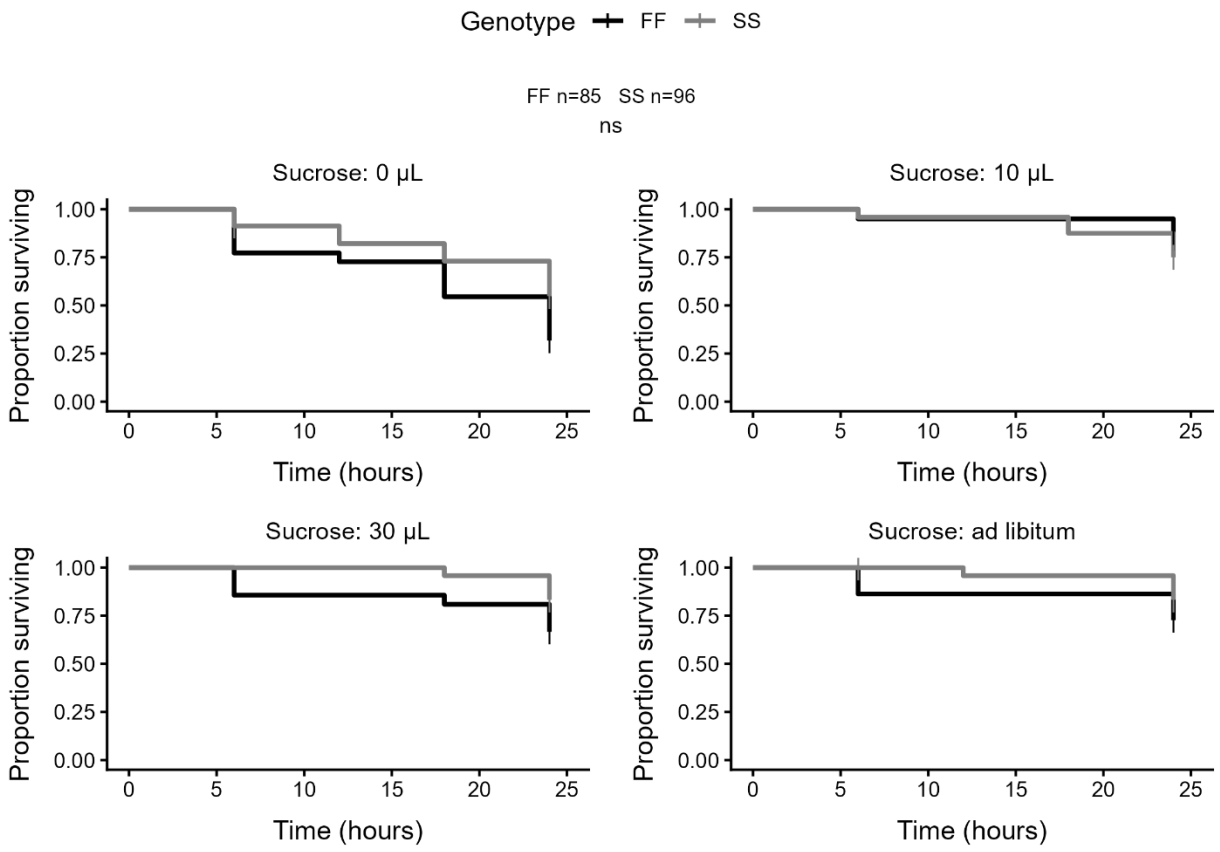

Figure 5a: Kaplan–Meier survival curves for FF and SS bees across sucrose doses (0, 10, 30 µL and ad libitum; FF: n = 85; SS: n = 96).

Log-rank tests show no statistically significant genotype contrast at any dose of sucrose (all p >0.11).

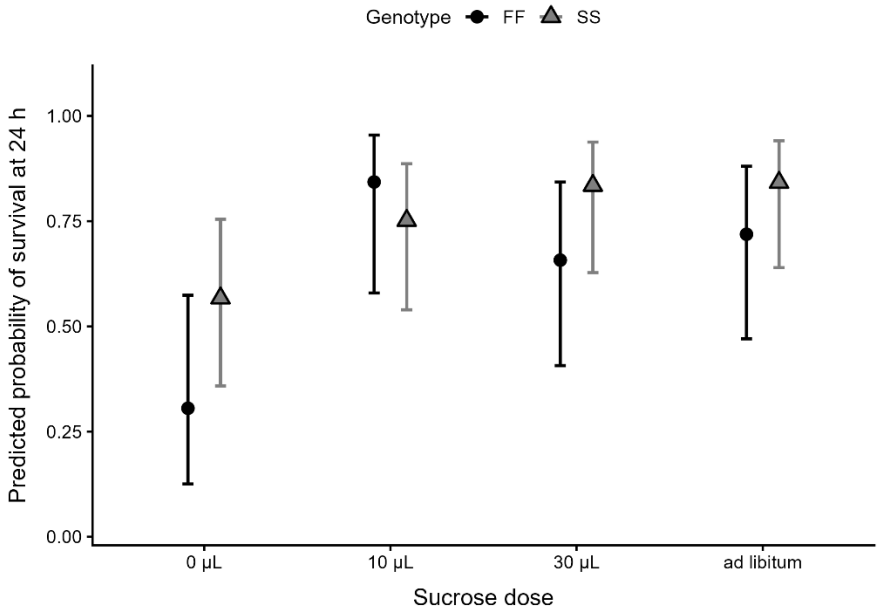

Figure 5b: Points represent model-predicted probabilities of survival at 24 h derived from a binomial generalized linear mixed model (GLMM) with a logit link. Error bars represent 95% confidence intervals derived from log-scale models and back-transformed for visualization. Survival increased with sucrose availability in both genotypes, with similar patterns observed for SS and FF bees. Sample sizes were SS = 96 and FF = 85 bees.

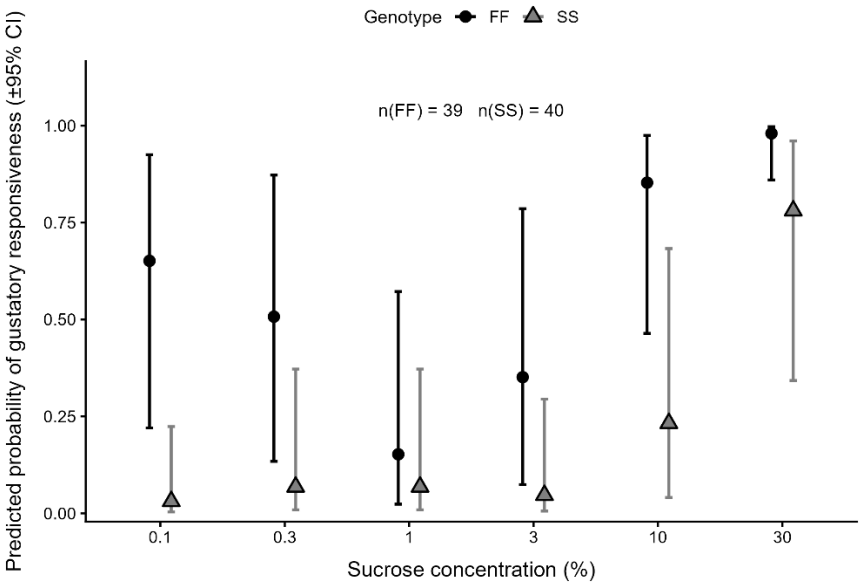

Figure 6. Gustatory responsiveness — model-predicted probabilities (GLMM):

Points represent model-predicted probabilities of gustatory responsiveness derived from a binomial generalized linear mixed model (GLMM) with a logit link and error bars represent 95% confidence intervals derived from log-scale models and back-transformed for graphing. FF bees were more responsive overall. Sample sizes were SS = 40 and FF = 39 bees.

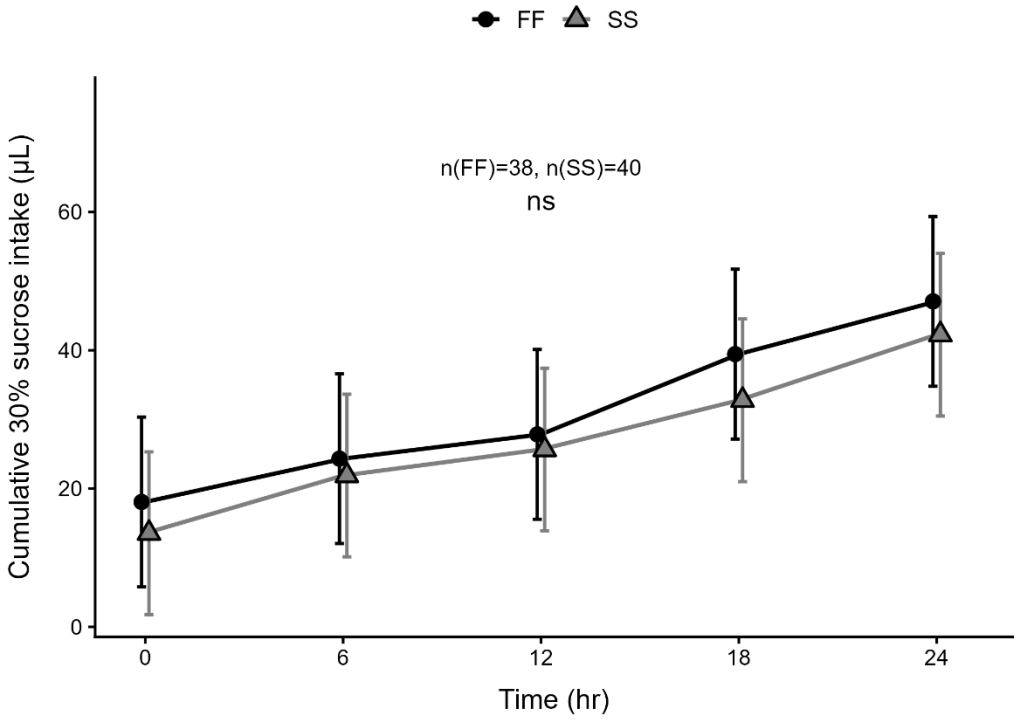

Figure 7a: Cumulative 30% sucrose intake across 24 hours.

Model-estimated mean cumulative sucrose consumption (µL) at 0, 6, 12, 18, and 24 h for SS (n = 40) and FF (n = 38) bees. Estimates are derived from a linear mixed-effects model including genotype, time, and their interaction as fixed effects, with Bee ID and source colony as random intercepts. Error bars represent 95% confidence intervals derived from log-scale models and back-transformed for graphing. Cumulative intake increased significantly over time, while no significant effects of genotype or genotype × time interaction were detected.

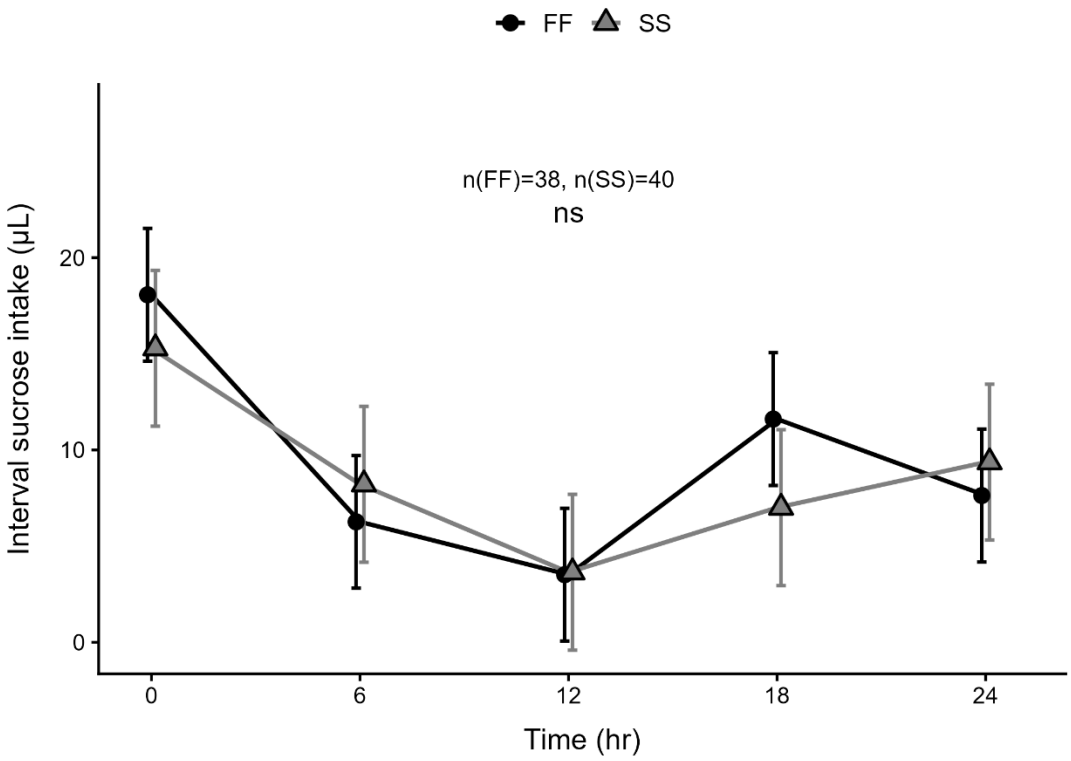

Figure 7b: Interval sucrose intake for SS and FF bees every 6 -hours

Model-estimated mean sucrose intake (µL) within successive 6-hour intervals (0–6, 6–12, 12–18, and 18–24 h) for SS (n = 40) and FF (n = 38) bees. Estimates are derived from a linear mixed-effects model with genotype, time interval, and their interaction as fixed effects, and Bee ID and source colony as random intercepts. Error bars represent 95% confidence intervals. Both genotypes showed similar temporal patterns of intake, with reduced intake during mid-intervals and partial recovery during the final interval.

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