Plasticity and subfertility at the evolution of eusociality: an empirical test of the evolution of role specialisation

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Introduction

The origin of eusociality, where some individuals gave up their own reproduction to help raise the offspring of others, represents one of the major evolutionary transitions (*1*). The key feature of eusociality is role specialization, where a reproductive caste (queen) specializes in egg production while a helper (worker) caste specializes in non-reproductive tasks such as provisioning offspring. The resulting division of labour is often further elaborated through the evolution of morphological and physiological castes (*2*). Various mechanisms that might initially have favoured helping have been proposed, including fitness insurance benefits and the unusually close relatedness among sisters as a result of haplodiploidy (*3–5*). However, empirical tests largely focus on species that evolved eusociality >20 Mya ago, making it difficult to determine whether role-associated traits evolved to enhance task performance after the transition to eusociality, or whether these traits were already present in non-social ancestors and facilitated the origin of sociality itself. Today's eusocial taxa may not be representative of the phenotypes present when eusociality first evolved, or of the payoffs that the first helpers could obtain (*6*). For example, even in primitively eusocial taxa (lacking morphological castes), queen fecundity typically increases linearly with the number of

helpers (*7*), but whether non-social females were sufficiently plastic to achieve this at the origin of sociality remains unknown.

A central tenet of life-history theory is that because resources are limited, investment in one aspect of life must be traded-off with investment in another (*8*). Before sociality evolved in both insects and vertebrates, investment in offspring production is likely to have been limited by resources available to provide the parental care necessary to successfully raise those offspring. Indeed, there is widespread evidence across non-social taxa for the trade-off between offspring production and parental care (*9*–*11*). At the origin of eusociality, this trade-off was partly or completely removed: reproductives no longer had to invest in parental care, and helpers no longer invested in reproduction. In order to understand how role specialisation facilitated or constrained eusociality at its origin, we must determine how plastic non-social females were when they first specialized. If relieving the allocation trade-off was sufficient alone to lead to the evolution of sociality, then two conditions must have been met: (i) a newly specialised queen must have been able to produce enough eggs to match the reproduction of herself and her helper(s) if they instead nested independently, and (ii) a newly specialised helper must have been able to collect enough provisions to feed those offspring. In addition, a feature of eusocial insects is the morphological differentiation among castes, where queens are often larger than workers. It has therefore been hypothesised that the initial helpers, at the origin of eusociality, were small “subfertile” females, who potentially had fitness gains from helping a larger, more fertile, relative (*12*). This morphological differentiation could further reduce the reproductive requirement of newly specialised queens because she would only need to produce enough extra eggs to replace the reproduction of her smaller, less fecund helper. However, whether such a helper would have been able to provide enough provisions for these offspring remains unknown. Here, we manipulate a potential proxy ancestor, enabling us to directly measure role plasticity and test whether any productivity benefits depend on the phenotypes of interacting females.

Results

Plasticity at the origin of eusociality

Wasps and bees are an ideal lineage in which to investigate the origin of eusociality. They include the full range of strategies from non-social (also known as subsocial) taxa, where each female nests alone and provisions her own offspring, to highly eusocial species with morphologically differentiated castes (*13*). Apoid digger wasps comprise several speciose lineages from within which eusocial bees and silk wasps evolved (*14*, *15*). We studied female *Ammophila pubescens*, a non-social apoid wasp where mothers produce successive nests each containing a single offspring, making the system unusually tractable (*10*). Offspring are provisioned progressively, as in almost all eusocial wasps. To produce an offspring, a female first digs a short burrow in sandy soil, ending in a single cell. She then hunts and paralyses a lepidopteran caterpillar and places it in the cell, laying a single egg. After typically 2-4 days, during which she may initiate other nests, the female returns, assesses whether her egg has hatched and if so, adds several (mean = 3.40 ± 0.19) further prey one at a time over a period of 1-7 days. She then seals the nest entrance and her larva consumes the prey and pupates (see Controls in Figure 1).

In order to measure role plasticity, we forced individually marked females to specialise throughout their life in either (i) egg-laying, by restricting provisioning or (ii) provisioning, by increasing provisioning demands, along with (iii) a Control treatment (Fig. 1). To induce egg-laying specialization, we aimed to destroy each nest as soon as the female had laid her egg, so that she provisioned only a single prey item. To induce provisioning specialization, we aimed to remove 7 out of every 8 prey items as females arrived at their nest, before she has chance to provision her offspring. Females were therefore forced to capture additional (replacement) prey items above the number captured by Controls. To determine whether greater specialisation leads to greater benefits, we also performed partial specialisation treatments by destroying only alternate nests for the egg-laying treatment and removing only alternate prey for the provisioning treatment.

Our manipulations, along with naturally occurring variation and occasional deviation from the intended treatment level, resulted in a continuous range of role specialisation, from individuals that specialised almost fully on egg-laying to individuals that specialised almost fully on provisioning. We found no difference in the provisioning rate between the individuals in our most extreme provisioning specialisation treatment and the moderate treatment ($p = 0.89$, Supplementary Table 1) and as such we assume all individuals across these two treatments were provisioning at their maximum rate.

We quantify a female's specialisation towards a role (hereby referred to as "role specialisation") as the mean number of prey items she captured per egg throughout her life, with higher values indicating specialisation towards provisioning and lower values indicating specialisation towards egg-laying. The treatments were carried out continuously throughout the lives of two cohorts of females in two consecutive field seasons (~8 weeks summers of 2024 and 2025).

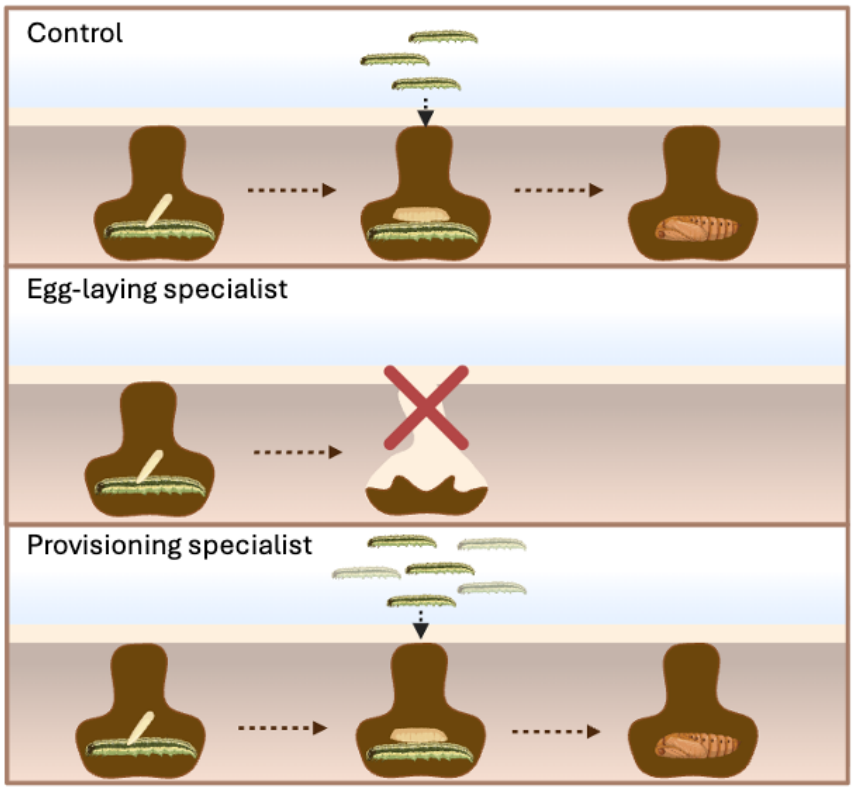

Fig. 1. ***Diagram of the experimental manipulation of egg-laying and provisioning behaviour.***
Control individuals were left to produce eggs and provision their young as normal. Egg-laying specialists had their nest destroyed after egg-laying. Provisioning specialists had prey (lepidopteran caterpillars) removed before the female could take it into the nest, resulting in her having to locate a replacement prey item and thereby increasing provisioning effort.

Our first finding was that productivity increases as specialisation increases. Females that specialised more on egg-laying (provisioning fewer prey per egg) had higher egg-laying rates than expected after accounting for body size, lifespan and cohort ($p < 0.001$, Supplementary Table 2, Figure 2). Similarly, those with greater specialisation towards provisioning had higher provisioning rates than expected ($p < 0.001$, Supplementary Table 2, Figure 2). However, for natural selection to favour specialisation through these benefits alone, the increase in productivity in the specialist role must at least match the degree of specialization. For example, a ≈100% increase in specialization, where a female gives up provisioning (or egg-laying) altogether, requires at least a 100% increase in egg-laying (or provisioning) rate to match the productivity of two females nesting independently. Our results instead imply a constraint: a 1% increase in egg-laying specialisation is associated with only a 0.73% increase in egg laying rate, while a 1% shift towards provisioning specialisation leads to only a 0.82% increase in provisioning rate, both smaller than required.

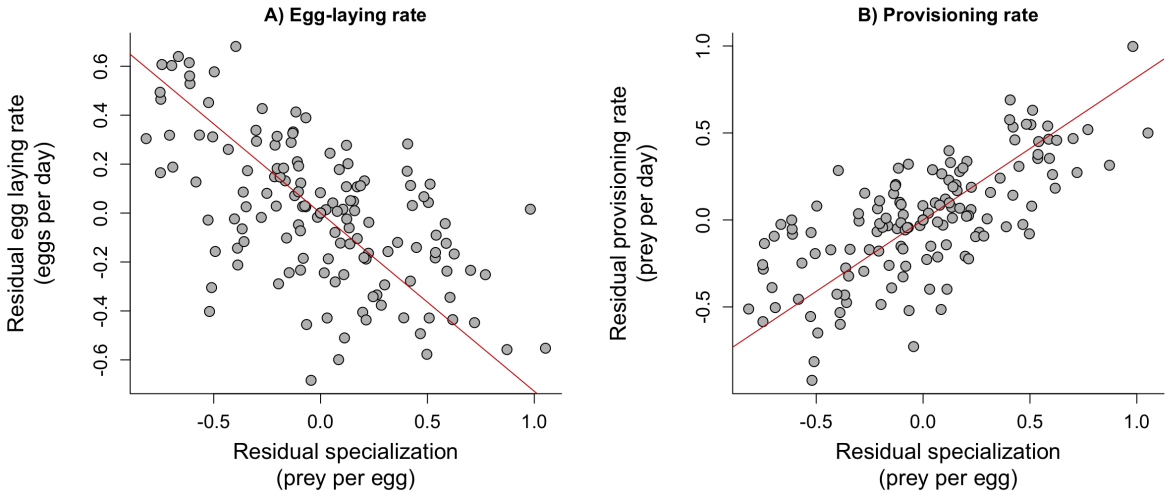

Figure 2: Correlations between A) residual egg-laying rate and residual role specialization, and B) residual provisioning rate and residual role specialization. Points represent individuals (n = 145) and the regression line is from the model estimates.

One way in which this constraint might be overcome is if greater specialisation leads to disproportionately larger benefits (*16*). As well as freeing up time and energy previously spent on activities now taken over by social partners, more extreme specialists might become more skilled at the given task, for example through learning or benefitting from no longer having to switch between tasks that require different physiologies (*17*, *18*). In our population, we found that there were increased benefits from specialising in egg-laying (p = 0.06, Supplementary Table 3). More extreme egg-laying specialists (residual values <0 of our 'role specialization' index) exhibited a 1.2% increase in their egg-laying rates for each 1% extra unit of specialization (p <0.001, Supplementary Table 3), whereas egg-laying increased by only 0.90% for residual specialisation values >0 (p = 0.008, Supplementary Table 3). In contrast, however, there was no evidence for increased benefits with provisioning specialisation (p = 0.76, Supplementary Table 3), even under our most severe manipulation, which was expected to increase provisioning effort eightfold, indicating that provisioning effort did not reach the levels targeted by this manipulation. Thus, while there might be sufficient plasticity for selection to favour specialist egg-layers, provisioning appears to be less plastic, perhaps because it is more costly than egg production (*10*). In social systems, helpers often also assist in nest building and maintenance, which we did not manipulate. This would tend to

accentuate our findings: additional resources used to build the nest would be freed up for egg-laying specialists, while provisioners would bear additional costs.

Subfertility at the origin of eusociality

In most organisms including Hymenoptera, offspring production is positively correlated with body size (*19*, *20*). The caste system in eusocial insects is often characterised by morphological differences, where large reproductives (queens) are helped by smaller, sterile workers which perform tasks such as foraging, defence and maintenance of the nest. These observations underlie the long-standing hypothesis that at the origin of eusociality, the first helpers were small, "subfertile" females that would be relatively unsuccessful nesting independently but could gain by helping a larger, more fertile relative to reproduce (*12*, *21*, *22*). Role specialisation might therefore evolve more easily if females can choose partners based on their condition (*21*).

The subfertility hypothesis focusses on variation in reproductive potential, but while small individuals may have low fertility, they may perform just as badly as helpers (*6*). At first sight, natural size variation in *A. pubescens* seems to confirm this: smaller Control females had lower provisioning rates ($p = 0.04$, Supplementary Table 4, Figure 3b) as well as lower egg-laying rates than larger females ($p = 0.004$, Supplementary Table 4, Figure 3a). However, because different components of care are normally inter-dependent (e.g. provisioning requires an egg to have been laid), small females might be capable of provisioning at a higher rate but be unable to express this because of constrained fecundity. Our manipulation relaxes the normal reliance on egg production for provisioning, allowing us to investigate how females of differing sizes perform when these components of care are differentially expressed. By comparing the Control females to those where we manipulated their provisioning to be maximised, we found no difference in the relationship between body size and provisioning rate ($p = 0.91$, Supplementary Table 5, Figure 3c). It is therefore the case that the smaller, less fecund females in our population do not appear to have any greater benefit of specialising in provisioning. However, it is still possible that small helpers could provision at a level to

support the reproduction of a specialist reproductive, even if she is still relatively poor at provisioning compared to other, larger individuals. Our manipulation, in turn, also allows us to consider various size pairings to determine whether there is the potential for complementary phenotypes in our population.

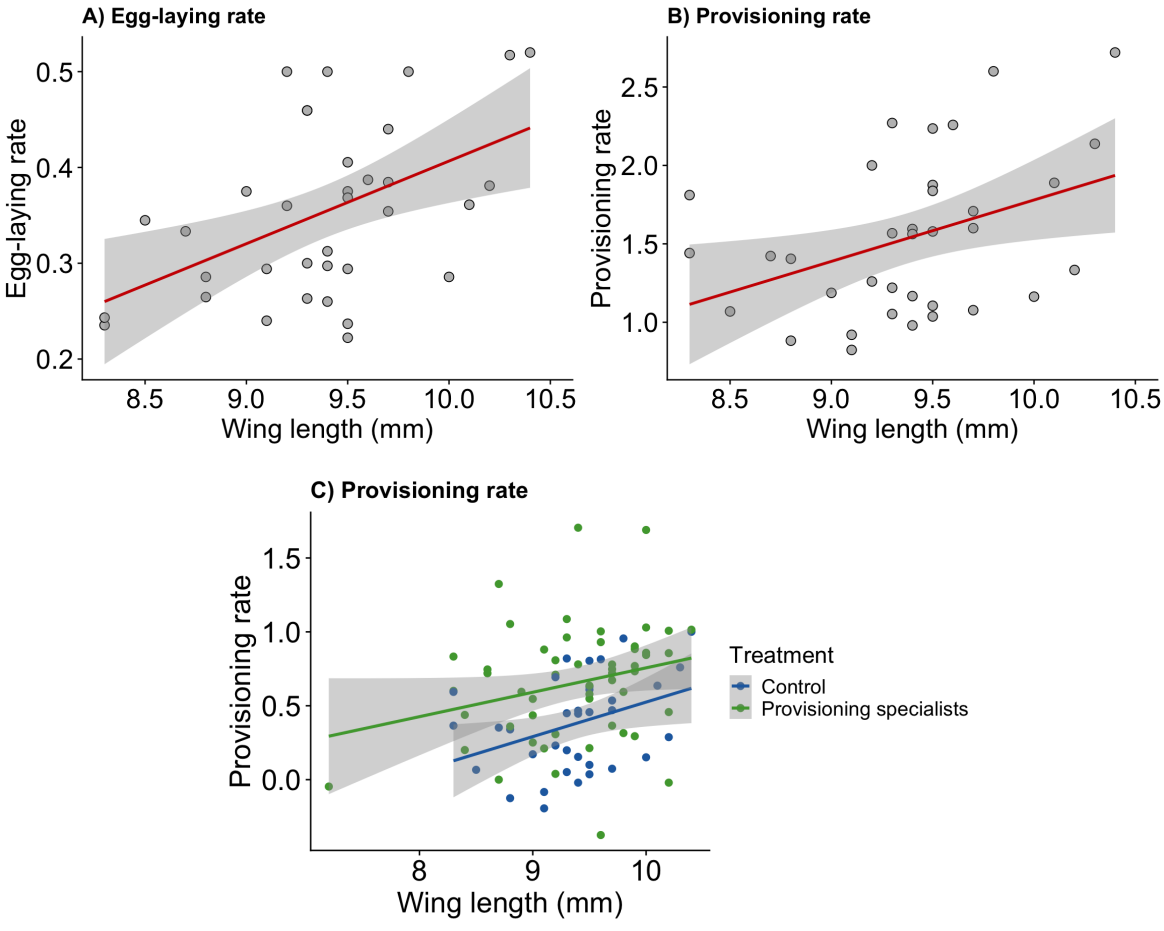

Figure 3: The relationship between wing length and A) egg-laying rate, B) provisioning rate for unmanipulated A. pubescens females (n = 35), and C) provisioning rate for

Control (n = 35) and provisioning specialist (n = 55) A. pubescens. The slopes are the regression lines, and the shaded regions are the 95% confidence intervals.

Methods

Study system

We monitored lifetime adult breeding of 145 females (64 in 2024 and 81 in 2025) in 2024 and 2025 at Witley Common, Surrey, United Kingdom (51º09'04'N, 00º40'53'W). Individuals were marked with unique colour marks so they could be identified and tracked throughout their adult lifespans. Each individual's body size was measured during marking by measuring wing length. All individuals' behaviour was monitored while at the breeding area (a ~10 x 3m section of bare soil) and while active (during daylight hours, with wasp activity stopping with substantial cloud cover). Each nest was uniquely marked and monitored until activity at the nest stopped. Egg laying was determined by the duration an individual was inside the nest with a newly captured prey (a female usually spends >25s in the nest for oviposition and <10 s for provisioning larvae).

Experimental manipulation of task specialisation

We manipulated egg-laying and provisioning behaviour to force a range of specialisation from egg-laying specialists to provisioning specialists (see Fig. 1). To do this we had five treatment groups; (i) extreme egg-laying specialists (n = 24), (ii) moderate egg-laying specialists (n = 31), (iii) control individuals (n = 35), (iv) moderate provisioning specialists (n = 27), and (v) extreme provisioning specialists (n = 28). Each individual was assigned a treatment randomly (maintaining equal variation in body size across treatment groups) after all individuals were marked.

Egg-laying specialisation was manipulated by either removing the egg after laying or destroying the nest, meaning the female never reaches the point of provisioning these offspring and instead, after attempting to inspect the nest, begins a new nest. Egg removal was performed by replacing the initial caterpillar with one tied with a tail of cotton, which after the female pulled in and oviposited on, could be pulled out of the nest. After an initial trial period, we swapped to exclusively destroying nests as individuals would frequently reject the replacement caterpillar and forage for a new prey. For extreme egg-laying specialists, we aimed to prevent provisioning after each egg was laid, with the intention that these individuals would never provision beyond the first caterpillar on which the egg was laid. For moderate egg-laying specialists, we aimed to prevent provisioning on half of nests throughout the breeding season, by alternating between preventing provisioning for a given nest and then letting provisioning happen as normal for the next nest.

Provisioning specialisation was manipulated once individuals began provisioning the larvae (i.e., after the inspection visit). Caterpillars could be removed while the female was opening the burrow, the female would then close the burrow and forage for another prey. In natural nests (with no manipulation), prey items are frequently stolen by other females or various ant species, and so removal of prey does not disturb their normal behaviour. This was also confirmed by comparing provisioning and egg laying between two control groups, one where provisioning occurred as normal and one where prey items were taken for ~1-2 s and then put back, and we found no significant differences between these groups. For extreme provisioning specialists, we aimed to remove 7 out of 8 prey brought to the nest, which should increase provisioning effort to close to the maximum level an individual is capable of (with unmanipulated nests being completed with an average of 4.40 caterpillars). For moderate provisioning specialists, we removed alternate prey brought to each nest, doubling the effort required to provision an individual's nest as normal.

Due to the nature of manipulations on wild animals, our treatment categories were imperfect (for example, due to nest failure, brood parasitism/provisioning another wasps nest, egg-laying specialists returning with prey to destroyed nests and missed

prey removal), which meant the specialisation in strategies could be treated as a continuous variable. We measured specialisation by quantifying the number of prey items per eggs laid, with lower values indicating specialisation in egg-laying and higher values indicating specialisation in provisioning.

Data analysis

Both the number of provisions and eggs laid were converted to rates (per day lived) to account for variation in lifespans among individuals. We extracted residual log egg-laying rate, residual log provisioning rate and residual log role specialisation for each individual from linear models with body size, year and lifespan as fixed effects.

We analysed the relationship between residual egg-laying rate and residual specialisation, and residual provisioning rate and residual specialisation in separate Standard Major Axis (SMA) regressions using the *lmodel2* package in R (R Development Core Team, 2009). To determine whether the benefits of specialisation are greater when individuals specialise, we compared the slopes of SMA regressions for above and below zero for both residual egg-laying rate and residual provisioning rate separately.

To determine whether body size affects an individual's ability to specialise in a given task, we used generalised linear models of both log egg-laying rate and log provisioning rate in separate models with a Gaussian distribution using the *stats* package in base R. Body size, lifespan and year were fixed effects in both models. Note, removing an outlier with a very small body size did not change the outcomes of our models and so remained in the dataset throughout our analyses.

All analyses were conducted in R version 4.4.0 (*24*).

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Supplementary Information

Supplementary Table 1: Model outputs for assessing the difference in provisioning rate between the two provisioning specialisation manipulation categories. Text in parentheses denotes the factor level contrasted with the reference level for categorical fixed effects. Significant values are presented in bold.

	Estimate	SE	p- value
Intercept	-1.06	0.86	0.22
Lifespan	-0.01	0.01	0.12
Body size	0.21	0.08	**0.02**
Treatment (extreme provisioning specialist)	-0.01	0.11	0.89
Year (2025)	0.11	0.14	0.42

Supplementary Table 2: The relationship between role specialization and productivity. Model outputs for a reduced major axis analysis testing the relationship between residual role specialization and (i) residual egg-laying rate, (ii) residual provisioning rate. Higher role specialization values indicate specialization towards provisioning and lower values indicate specialization towards egg-laying. Zero indicates no directional specialization, with both provisioning and egg-laying equal to the median strategy in the population. Significant values are presented in bold.

Model	Slope (95% CI)	r^2	p-value
Residual egg laying rate vs residual specialization	-0.73 (-0.83 - -0.64)	0.35	**<0.001**
Residual provisioning rate vs residual specialization	0.82 (0.73 – 0.93)	0.48	**<0.001**

Supplementary Table 3: Benefits of more extreme specialization. Model outputs for reduced major axis analysis comparing rates of increase (slopes) in productivity in the specialist role as specialism becomes gradually more extreme when (i) our role specialization index is low (residual prey per egg < 0, increasing egg-laying specialism) or (ii) high (residual prey per egg > 0, increasing provisioning specialism). Between slopes p-values test whether the two slopes differ (i.e., evidence of increased benefits of more extreme specialization), and within slope p-values test whether slopes differ significantly from zero. Significant values are in bold.

Model		Between slopes p-value	Slope (95% CI)	r^2	Within slope p-value
(i) Egg-laying rate	Low residual egg laying rate vs prey per egg	**0.06**	-1.20 (-1.49 – 0.97)	0.21	**< 0.001**
	High residual egg laying rate vs prey per egg		-0.90 (-1.12 - -0.72)	0.10	**0.008**
(ii) Provisioning rate	Low residual provisioning rate vs prey per egg	0.76	1.17 (0.93 – 1.45)	0.15	**0.001**
	High residual provisioning rate vs prey per egg		1.11 (0.93 – 1.34)	0.42	**< 0.001**

Supplementary Table 4: Model outputs for predicting (i) log egg-laying rate and (ii) log provisioning rate given variation in body size for Control females (n = 35). Text in parentheses denotes the factor level contrasted with the reference level for categorical fixed effects. Significant values are in bold.

Model		Estimate	SE	p-value
Egg-laying rate (log)	Intercept	-3.21	0.78	**<0.001**
	Body size	0.24	0.08	**0.004**
	Lifespan	0.00	0.00	0.32
	Year *(2025)*	0.07	0.09	0.43
Provisioning rate (log)	Intercept	-1.59	1.05	0.14
	Body size	0.23	0.10	**0.04**
	Lifespan	-0.01	0.01	0.30
	Year (*2025)*	0.09	0.13	0.50

Supplementary Table 5: Model outputs for estimating the difference in the relationship between body size and provisioning rate between Control individuals (n = 35) and provisioning specialists (n = 55). Text in parentheses denotes the factor level contrasted with the reference level for categorical fixed effects. Significant values are in bold.

	Estimate	SE	p-value

Intercept	-1.49	1.14	0.20
Lifespan	-0.01	0.00	0.06
Body size	0.22	0.12	0.06
Treatment (provisioning specialist)	0.41	1.30	0.76
Year (2025)	0.10	0.09	0.26
Body size : Treatment (provisioning specialist)	-0.02	0.14	0.91