



## OPEN Spider predatory aggressiveness exhibits diverse personality and plasticity associations and complex neurophysiological mechanisms

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Although personality has been popularly studied for decades, the relationship between plasticity and personality remains unclear, and the underlying neurophysiological mechanisms are still not well understood. Here, we addressed this gap by comprehensively integrating field surveys, behavioral tests, transcriptome, and biochemical analyses to study the personality and plasticity of predatory aggressiveness in the wolf spider, *Hippasa holmerae*. A total of 103 *H. holmerae* were collected from a field population and their willingness to attack a dangerous East Indian harvest ant prey (*Carebara diversa*) was each evaluated. We found that nearly half of the individuals exhibited high behavioral plasticity while the others exhibited apparent personality, and results of modeling approach suggests that proactive personality might indeed limit adaptive plasticity. The brains of individuals displaying apparent high or low aggressiveness were subjected to transcriptome analyses. The results showed that 19 physiological systems had more than 20% of their associated genes exhibiting differential expression patterns between the high and low aggressive groups. Furthermore, almost all of these differences were due to genes being significantly upregulated in highly aggressive individuals. Results of biochemical analyses further showed that the concentration of tryptophan in the brains of highly aggressive individuals was significantly lower than that of low aggressive ones. These gene expression and amine variation patterns were consistent with the characteristics of big and small engine individuals proposed by the pace of life syndrome theory and energy management models. Here, we show the personality-plasticity association within a population and empirically demonstrate that personality differences might be caused by the differences in multiple physiological systems. These results highlight the importance of distinguishing personality and plasticity in behavioral tests and the importance of comprehensively examining multiple systems while conducting personality mechanistic studies.

**Keywords** Predatory aggressiveness, Personality, Plasticity, Transcriptome, Wolf spider

Personality, the repeatable and consistent individual behavioral variation within species that widely exists across animals<sup>1,2</sup>. Variation in personalities among individuals represents a form of intra-population behavioral diversity, which may contribute to population adaptability under varying environmental conditions<sup>3</sup>. The diversity of personalities can be viewed as a form of consistent phenotypic variation, with both genetic and environmental factors influencing personality development. For example, factors such as environmental enrichment<sup>4</sup>, social contexts<sup>5</sup>, single nucleotide polymorphisms (SNPs) in specific genes<sup>6</sup>, or additive genetic variations<sup>7</sup> have been shown to influence personality development. In recent years, an increasing number of animal species and even microorganisms have been reported to exhibit personality variation<sup>8–10</sup>, and relevant phenotypic traits examined include boldness<sup>11</sup>, aggressiveness<sup>12</sup>, and directional preference<sup>13</sup>.

Plasticity, how an individual responds after experiencing physical or social environmental changes caused by biotic or abiotic factors<sup>2</sup>, is another important phenotypic trait that could affect fitness. Plasticity enables particular behaviors of an individual to exhibit lower consistency and repeatability depending on the context<sup>14–16</sup>. Currently, the relationship between personality and plasticity is still poorly understood, and how

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they interact to influence the behaviors of individual animals seems quite complex. The effects of personality and plasticity may be positively or negatively correlated depending on different personality types, animal species, and environmental contexts<sup>2,17–20</sup>.

Studies on animal personalities can be grouped into two categories. The first focuses on how genetic, physiological or environmental factors<sup>4,6,11,21,22</sup> mechanistically influence the development of personalities. The second examines how personality variation functionally affects sexual selection<sup>23</sup>, natural selection<sup>24</sup>, or evolution<sup>25</sup>. Enhancing our understanding of the mechanistic and functional aspects can help integrate personality into ecological models to improve predictability and application potential. For example, some previous studies incorporated personality into predator-prey interaction models to predict consequences in simplified tri-trophic systems<sup>26,27</sup>. Therefore, a better comprehension of personality can enhance our understanding of the evolution of behaviors. It is believed that to fully understand personality, both mechanistic and functional aspects of animal behavior need to be integrated<sup>28,29</sup>.

Aggressiveness can be defined as the individual's consistent tendency to exhibit confrontational or hostile responses toward predators, prey, and conspecifics<sup>30</sup>, which is a type of proactive personality. Currently, hypotheses integrating mechanistic and functional aspects of aggressive personality are limited, with the pace-of-life syndrome hypothesis (PSH) being one example. The PSH proposes that physiological differences underlie observed personality variations. Within a population, there might be “high-metabolic individuals” with high energy demands exhibiting a more agonistic personality to obtain more energy. Therefore, these individuals should have higher fitness in resource-rich environments. Conversely, there are “low-metabolic individuals” with low energy demands exhibiting a less agonistic personality. Such individuals may have higher fitness in resource-poor environments<sup>31–36</sup>. We propose that by examining aggressive personality through transcriptomic and biochemical approaches, we can uncover how multiple physiological systems are interactively involved in shaping an individual's personality to empirically verify hypotheses such as PSH.

In this study, we aimed to determine the relationship between personality and plasticity by measuring willingness to attack using behavioral assays performed in a predatory aggressiveness context. We anticipated that some individuals would exhibit behavioral patterns more aligned with plasticity, while others would display patterns more characteristic of personality. In a well-designed predatory aggression test system, we subjected wolf spiders *H. holmerae* to interactions with a dangerous prey, the harvest ant *C. diversa*. We estimated the attack willingness level of each wolf spider towards the ants and the respective effects of personality and plasticity were identified and separated using a model building approach.

We analyzed predatory aggressiveness mechanistically with transcriptomic and biochemical approaches to explore the physiological differences underpinning variation in aggressiveness among individuals. We extracted total RNA from the prosoma of individual *H. holmerae* spiders to explore their neurophysiological differences using transcriptomic approaches. Gene expression patterns from the constructed libraries were verified and physiological systems exhibiting large gene expression differences between spiders with divergent aggressiveness levels were identified. Transcriptomic analyses revealed that between individuals with different aggressive levels genes associated with 19 physiological systems showed differential expression. These systems were related to signal transduction, signal transmission, memory/learning, sensory perception, venom secretion, and circadian rhythms. Such patterns are consistent with the predictions of the pace-of-life syndrome hypothesis<sup>31–36</sup>, which proposes that individuals with a high aggressive personality (i.e., “big-engine” individuals) tend to have enhanced physiological capacities. In addition, given that our transcriptome analyses revealed substantial differences in amine-related gene expressions between individuals with different aggressiveness levels, and considering that amines serve as key neural signaling molecules in regulating arthropod aggressive behaviors<sup>37</sup>, we further investigated whether these molecular differences were reflected in the actual amine concentrations (i.e., the transmitters themselves). We found that the tryptophan concentration was significantly lower in individuals exhibiting high aggressiveness, which is consistent with the results of previous studies<sup>37–39</sup>.

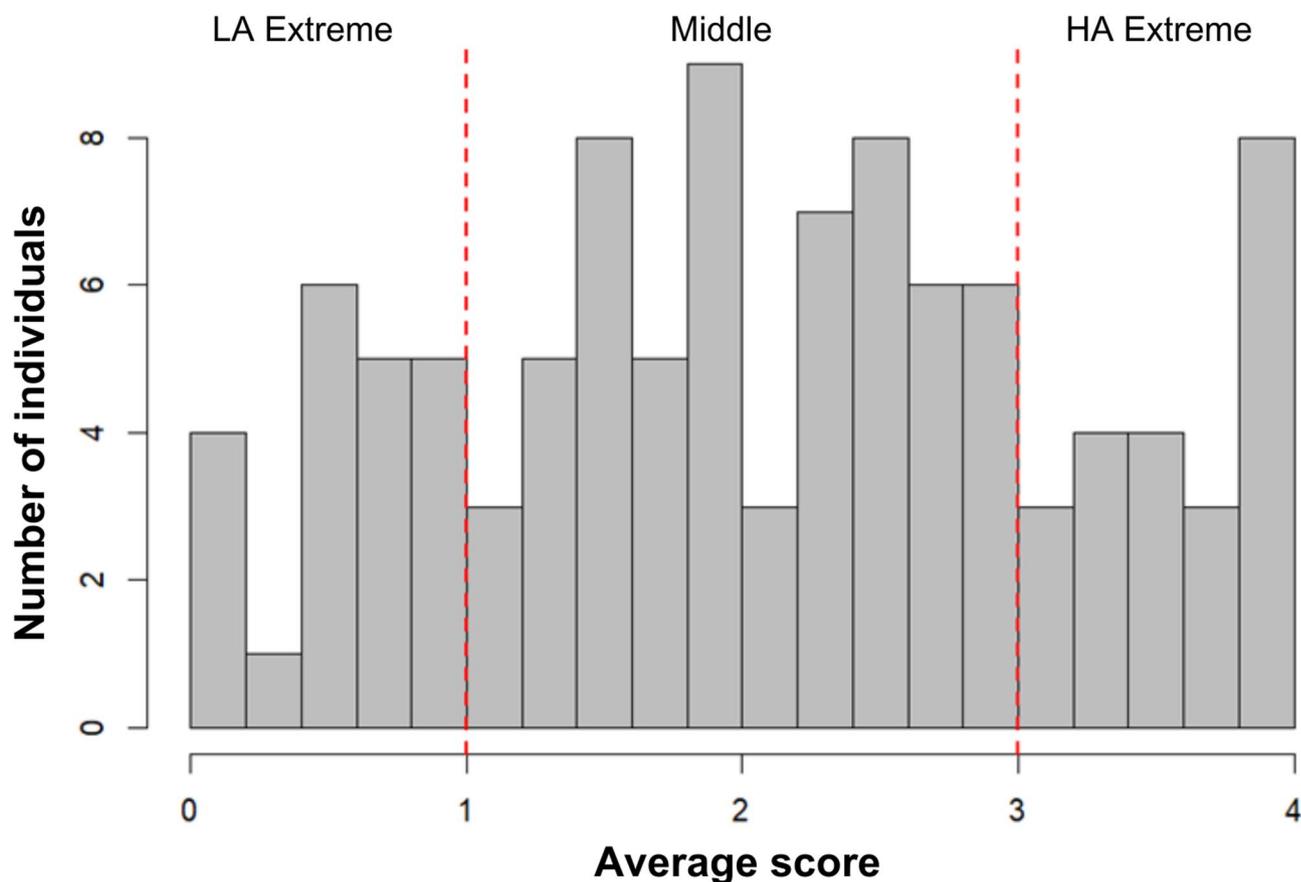
## Result

### Aggressiveness level of spider individuals examined

In this study we assessed the aggressiveness of individual wolf spider *H. holmerae* ( $n = 103$ ) by measuring level of willingness to attack a highly risky but low-value prey, the East Indian harvest ants *C. diversa*. We allowed *H. holmerae* to build a funnel web in an arena, introduced *C. diversa*, recorded their predatory interactions, and determined level of aggressiveness (from 0 to 4 points) according to a scoring scheme (Table 1). For each individual spider this procedure was repeated five times and the average score of five trials was used to indicate the willingness to attack. At the end of the behavioral tests there were 22 individuals (21.4%) with a mean score lower than 1 point and we assigned them to the LA (Low Aggressiveness) group. There were 28 individuals (27.2%) with a mean score higher than 3 points and we assigned them to the HA (High Aggressiveness) group. There were 53 individuals (51.5%) with mean scores between 1 and 3 points and we assigned them to the middle (Middle Aggressiveness) group. We designated LA and HA groups as the extreme groups (Fig. 1). During the behavioral tests, we discovered that overall, as the trials went on, the percentage of 4-point incidents decreased from 52.4 to 28.2%. In contrast, the percentages of 0 and 1-point incidents increased from 15.5% and 11.7% to 23.3% and 20.4% respectively, exhibiting a negative correlation between trial numbers and aggression level (Fig. 2a). More specifically, aggression levels of middle group individuals declined as trials went on suggesting low levels of repeatability in willingness to attack (Fig. 2b). In contrast, the behaviors of both HA and LA group individuals showed a highly repeatable pattern. Their behavioral test scores were more or less consistent throughout the five trials (Fig. 2c and d).

Behavioral Sequences		Score	
Exploring	Giving up		0 point
	Biting	Giving up	1 point
		Watching	2 point
		Dragging	4 point
	Multiple biting	Giving up	2 point
		Watching	3 point
		Dragging	4 point
	Dragging	4 point	

**Table 1.** The scheme of all conceivable sequences of hunting behaviors and their corresponding scores. The spider must exhibit initial exploring behavior to indicate awareness of the prey on its web; otherwise, the behavioral test would be restarted two days later. Dragging was considered the most aggressive and risky predatory behavior, and therefore individuals performing such a behavior were given 4 points.

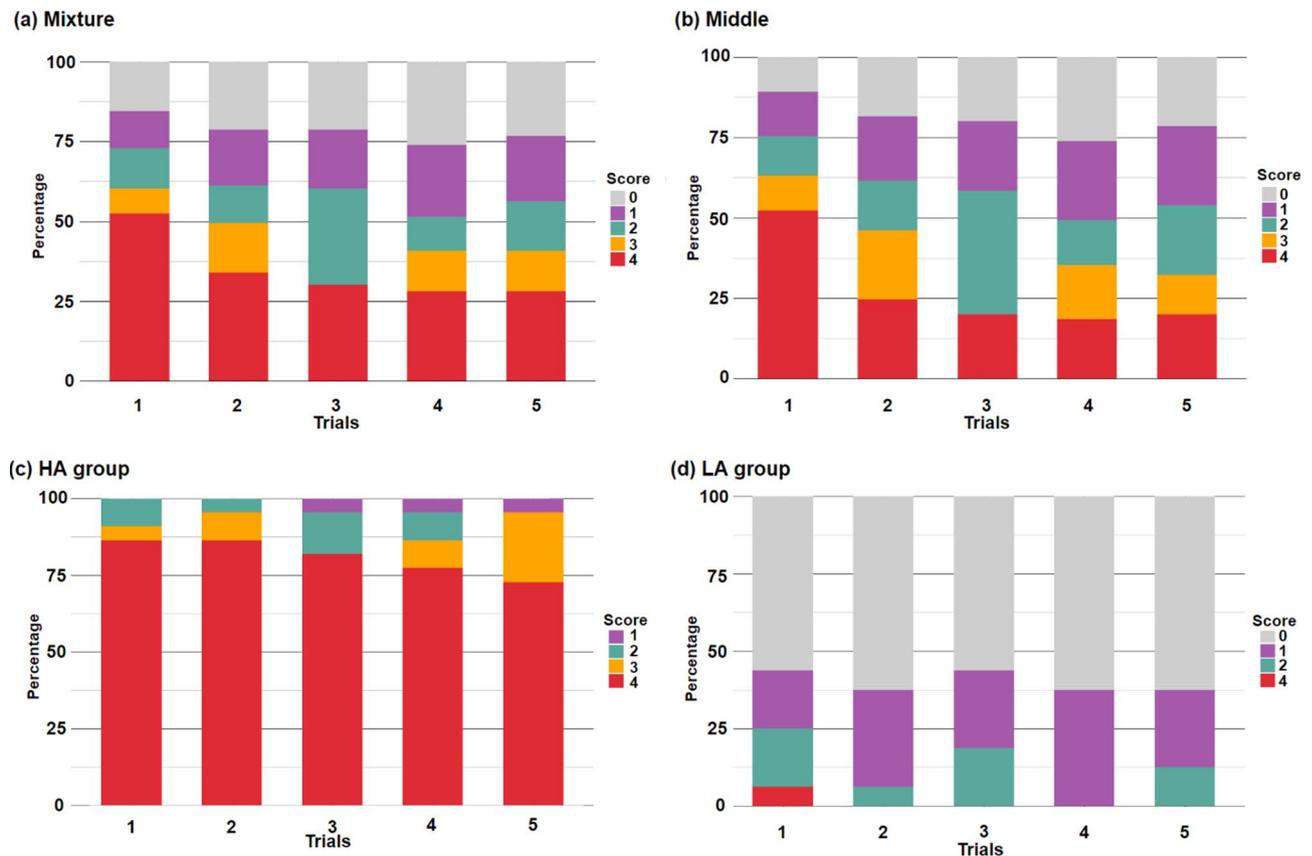


**Fig. 1.** Results of behavioral tests showing number of individual *Hippasa holmerae* exhibiting various mean scores. Individuals between dotted lines belong to the middle group, others belong to either HA (High Aggressiveness) or LA (Low Aggressiveness) extreme groups.

### Plasticity-personality association patterns of spiders examined

We employed a model-building approach to analyze how personality and plasticity affected spider predatory aggression and their association. As previously described, we divided all 103 spiders into three groups based on their behavioral performance: HA, LA, and middle group (Fig. 1). To analyze the impact of personality and plasticity and their interaction on willingness to attack, we built the best-fitting generalized linear mixed models (GLMM) or generalized linear models (GLM) to describe the behaviors of all spiders combined (mixture), intermediate individuals alone (middle), and HA and LA individuals alone (extreme). The results of these models assessing the personality–plasticity association in the three groups are summarized in Table 2.

Spider ID was used as a random factor to represent personality, and trial number was treated as a fixed factor to represent plasticity. Trial number was analyzed both as a categorical variable (each trial compared to the



**Fig. 2.** Percentages of individuals exhibiting various aggressiveness scores in five trials. (2a) While we took all 103 individuals together, initially over 50% of individuals exhibited highly aggressive 4-point behavior. However, by the fifth behavioral trial, only about 25% showed this behavior. An opposite pattern was observed in 0-point and 1-point aggressive behaviors, indicating that most spiders progressively reduced their aggressiveness as the trials continued, while a small proportion maintained extremely high or low aggressiveness. (2b) The middle group showed a pattern similar to the mixed group, with a clear decline in aggressive behavior as the trials progressed, demonstrating low repeatability in their predatory aggressive behaviors. (2c) In the HA (High Aggressiveness) group, no 0-point incidents were observed, and most individuals showed highly repeatable predatory aggressive behaviors. (2d) In contrast, most individuals in the LA (Low Aggressiveness) group consistently displayed low levels of predatory aggressive behavior, with only one instance of 4-point behavior observed in trial 1.

baseline trial 1) and as a numerical variable (representing a linear trend from trial 1 to 5). Initially, we built full models incorporating three factors: personality (spider ID as random factor), plasticity (trial number as fixed factor), and their interaction ( $ID \times trial\ number$ ). However, models including the interaction term failed to converge and showed increased AIC values, indicating that simpler models without the interaction term could best describe our data.

For the mixture model, the best fit was achieved using a GLMM including both fixed and random effects. When trial number was treated as a categorical variable, scores from trial 2 to trial 5 all showed significant declines compared to trial 1. When trial number was treated as a numerical variable, we observed a significant negative correlation between trial number and behavioral scores, consistent with the trend shown in Fig. 2.

For the middle model, a GLM with the fixed effect alone best described the data. When trial number was analyzed categorically, scores from trial 2 to 5 again showed significant declines. When being modeled as a numerical variable, trial number exhibited a significant negative correlation with behavioral scores. In contrast, including a random effect for spider ID in a GLMM resulted in a drop in random effect variance from 0.19 to 0, triggering a singularity warning and increasing the AIC to 935, suggesting that the random effect was negligible in this group.

In the extreme model, a GLMM with only the random effect of spider ID yielded the lowest AIC (858.9), indicating a strong personality signal. However, we found that the fixed effect of trial number remained detectable and meaningful. Therefore, we ultimately adopted a GLMM including both fixed and random effects (AIC = 862.3) to illustrate this trend. When trial number was treated categorically, no significant differences were found between trials. The random effect variance was higher than in the mixture model (from 0.19 to 0.573), highlighting the increased consistency in behavior. However, when trial number was treated as a numerical variable, it was significantly negatively correlated with behavioral scores, although the p-value ( $p = 0.038$ )

Model (trial number as categorical)	Term	Estimate	Z value	P
Mixture (GLMM)	Trial2	-0.19	-2.148	<b>0.032</b>
AIC = 1841.9	Trial3	-0.305	-3.342	<b>&lt; 0.001</b>
	Trial4	-0.33	-3.587	<b>&lt; 0.001</b>
	Trial5	-0.29	-3.196	<b>0.001</b>
Variance of random factor = 0.19				
Middle (GLM)	Trial2	-0.265	-2.111	<b>0.035</b>
AIC = 933.28	Trial3	-0.44	-3.33	<b>0.001</b>
	Trial4	-0.43	-3.26	<b>0.001</b>
	Trial5	-0.339	-2.644	<b>0.008</b>
Extreme (GLMM)	Trial2	-0.112	-0.817	0.369
AIC = 862.3	Trial3	-0.173	-1.624	0.172
	Trial4	-0.229	-1.966	0.076
	Trial5	-0.238	-1.558	0.065
Variance of random factor = 0.573				
Model (trial number as numerical)	Term	Estimate	Z value	P
Mixture (GLMM)	Trial	-0.076	-3.622	<b>&lt; 0.001</b>
AIC = 1840.8				
Middle (GLM)	Trial	-0.092	-3.037	<b>0.002</b>
AIC = 933.65				
Extreme (GLMM)	Trial	-0.061	-2.072	<b>0.038</b>
AIC = 856.7				

**Table 2.** Results of analyses using generalized linear mixed models (GLMM) and generalized linear model (GLM) with Poisson distribution to assess the personality-plasticity association in *Hippasa holmerae*. Spider ID was treated as a random factor to represent personality, and trial number was treated as a fixed factor to represent plasticity. Trial number was analyzed both as a categorical variable (each trial compared to baseline trial 1) and as a numerical variable (linear trend from trial 1 to 5). **Mixture:** included all individuals and was best described by a GLMM with both fixed and random factors. **Middle:** included only intermediate-aggressiveness individuals and was best described by a GLM with only fixed factor. Notably, when being modeled using a GLMM, the variance of the random effect was estimated as zero. **Extreme:** included only High-Aggressiveness (HA) and Low-Aggressiveness (LA) individuals and was optimally described by a GLMM containing only the random factor (spider ID; AIC = 858.9); however, the results shown here also include the fixed factor to demonstrate that the effect of plasticity remains detectable among extreme groups.

was higher than that of the middle model ( $p = 0.002$ ). This suggests that the fixed effect (plasticity) remained detectable, although weaker.

Finally, both extreme and middle models exhibited higher AIC values compared to mixture, suggesting that these two groups indeed showed a different behavioral pattern. Together, this information indicated a high level of plasticity behavioral pattern (i.e. low consistency) in individuals of middle group, and a high level of personality behavioral pattern (i.e. high consistency) in individuals of extreme groups.

### Gene expression patterns of spiders with different personalities

We used the prosoma of three extremely aggressive individuals with a mean score higher than 3.5 points and three extremely non-aggressive individuals with a mean score lower than 0.5 point for RNA extraction and subsequent transcriptome analyses. After library construction, sequence annotation and quantification, results of transcriptome analyses showed that sequences from 125,377 genes were obtained and among them 7,588 were DEGs. Due to the lack of *H. holmerae* genetic information, a total of 85,010 genes could not be identified by BLASTX. The volcano plot constructed from all the genes showed that the expression pattern of many genes differed considerably in spider individuals of HA and LA groups (see Figure S1 of Supplementary Information 1). Results of KEGG pathway analyses showed that in three signal transduction systems, one signal transmission system, five amine systems, three sensory systems, three learning and memory-related systems, one salivary secretion system and three circadian rhythm systems more than 20% of relevant genes differed in expression pattern between the LA and HA groups (Table 3). Moreover, in these 19 systems almost all differences were due to relevant genes being significantly up-regulated in HA group individuals.

### Amine concentrations of spiders with different personalities

Since the results of transcriptome analyses revealed significant expression differences in genes associated with amine-related systems between HA and LA spiders, we further investigated whether these molecular differences would manifest in actual amine concentrations. We used high performance liquid chromatography (HPLC) to compare the concentration of amine-related chemicals in *H. holmerae* exhibiting high and low aggressiveness ( $n = 6$  each). After HPLC analyses, a mass spectrometer was used to quantify and verify the identity of chemicals.

System	Percentage of Difference	Number of up-regulated	Number of down-regulated
<b>Signal transduction</b>			
cAMP pathway	26.7%	35	4
Wnt pathway	20.59%	20	1
Calcium signaling pathway	20.57%	26	3
<b>Signal transmission</b>			
Synaptic transmission	40%	16	0
<b>Signal transmission: Amine system</b>			
Dopaminergic synapse	25.3%	19	2
GABAergic synapse	24.62%	15	1
Serotonergic synapse	23.81%	19	1
Glutamatergic synapse	23.53%	19	1
Cholinergic synapse	20.45%	17	1
<b>Sensory</b>			
Phosphotransduction – fly	50%	8	1
Olfactory transduction	40%	9	1
Taste transduction	20%	7	1
<b>Memory &amp; Learning</b>			
Long-term potentiation	34.88%	14	1
Long-term depression	26.67%	11	1
Neurotrophin signaling pathway	20%	18	0
<b>Venom</b>			
Salivary secretion	42.86%	22	5
<b>Circadian rhythm</b>			
Circadian rhythm	27.27%	6	0
Circadian rhythm – fly	25%	2	0
Circadian entrainment	25%	17	2

**Table 3.** Results of KEGG analysis showing systems with more than 20% of relevant genes differed in expression patterns between HA (High Aggressiveness) and LA (Low Aggressiveness) groups. Up-regulated refers to genes significantly overexpressed in HA group compared to LA group, while down-regulated indicates the opposite. It is noteworthy that the distinctions within systems primarily stem from genes highly expressed in the HA group. Overall, there is a consistent trend of genes being significantly up-regulated in HA group compared to LA group.

Subsequently Student t-tests were used to compare the concentration of amine-related chemicals in spiders exhibiting high and low aggressiveness. Due to the unexpected low concentration of chemicals in spiders, only histamine, acetylcholine, glutamate, and tryptophan were successfully detected and quantified (Fig. 3). The first three chemicals did not show significant concentration differences between LA and HA individuals ( $t = -1.469$  for histamine;  $1.24$  for acetylcholine;  $-0.809$  for glutamate; all  $p > 0.05$ ) (Fig. 3a, b, c). However, the mean concentration of tryptophan in the LA group spiders was four times higher than that of HA group spiders ( $t = 3.299$ ,  $p = 0.021$ ) (Fig. 3d).

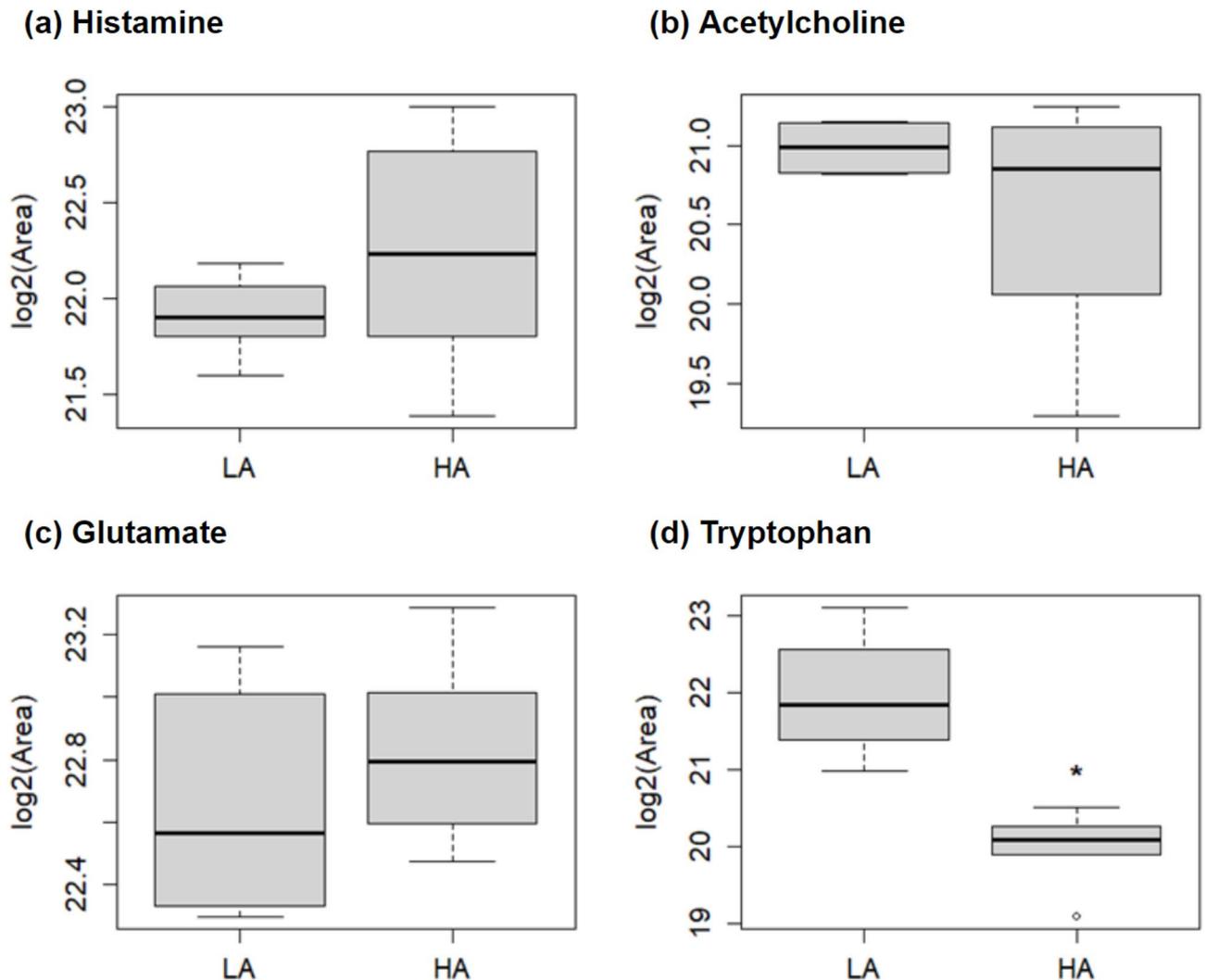
## Discussion

### Association between personality and plasticity

By controlling living environments, diet, sex and age, we found significant differences in the effects of plasticity and personality on aggressiveness between middle and extreme group spiders. To investigate these differences, we applied a modeling approach based on our predefined grouping, using the trial number as a probe of plasticity and the variance of the random factor as a probe of personality. This approach revealed that spider behavioral performance was better described when the middle and extreme groups were modeled separately.

All models showed that spiders consistently decreased their aggressiveness after repeatedly encountering high-risk and low-reward prey. Although we did not directly measure the fitness consequences of this behavioral change (e.g., injury rates), this behavioral plasticity may be adaptive, as it could help spiders minimize injury risks when confronting potentially dangerous prey with low nutritional value. This adaptive plasticity hypothesis is further supported by the observation that models for the mixture, middle, and extreme groups all showed a consistent and statistically significant decline in aggressiveness when trial number was treated as a numerical variable.

Moreover, when trial number was treated as a categorical variable, all three models showed relatively higher p-values for trial 2, suggesting limited behavioral differentiation at this early stage. Notably, in the extreme model, trial 3 also showed a relatively high p-value, indicating that behavioral differences in high-personality



**Fig. 3.** Amine-related chemical differences between HA (High Aggressiveness) and LA (Low Aggressiveness) groups. Four amine-related chemicals in HA and LA group spiders detected by HPLC analyses. The Y-axis represents the concentration (represented by peak areas in mass spectrometry) on a log<sub>2</sub> scale. Significant concentration difference was only found in tryptophan. (\*:  $p < 0.05$ ).

individuals emerged more slowly. These results highlight the importance of conducting multiple repeated trials in personality assessments: clear distinctions between high-plasticity (middle group) and high-personality (extreme group) individuals only became evident after trial 3. We therefore suggest that at least five repeated trials may be required to determine whether an individual's behavior is more determined by personality or plasticity.

Our model building analyses revealed distinct patterns when examining different subgroups of spiders. In the numerical models, the  $p$ -value for trial number in the extreme model was higher than that in the middle model, indicating a weaker effect of plasticity. Moreover, when trial number was treated as a categorical variable, a significant relationship with aggressiveness was found only in the middle group. Notably, when the middle group was modeled using a GLMM, the variance of the random effect was estimated as 0, together suggesting extremely low repeatability (i.e., personality) and showing high plasticity. In contrast, when only HA and LA individuals were included (model-extreme), the significant relationship between trial number and aggressiveness disappeared in the categorical model, while the variance of the random effect increased to 0.573. Taken together, these results indicate that predatory aggressiveness in the extreme groups was primarily determined by personality rather than plasticity, regardless of whether individuals displayed very high or very low aggressiveness.

The results of behavioral tests also helped us to have a glimpse at the relationships between proactive personality and adaptive plasticity. The pattern of plasticity in willingness to attack in the middle group suggests that these spiders may decrease their level of aggressiveness to prevent injury as trials progressed. In the numerical model, HA individuals also showed a weaker but still significant decline in average aggressiveness. According to the categorical model, this decline was mainly driven by lower aggressiveness in trials 4 and 5. This finding was consistent with the hypothesis proposed by previous studies that proactive personality limits

adaptive plasticity<sup>2,18</sup>, as the HA group spiders seemed to retain some level of adaptive plasticity, their response may be delayed or attenuated compared to middle group spiders.

Our research demonstrated that not all individuals in a population exhibited a distinct behavioral pattern that could be typically classified as personality, a phenomenon rarely explored in previous studies but with important implications for future research. In our study, over half of the individuals, primarily those in the middle group, did not display a clear personality. Based on the results of the behavioral tests, we hypothesize that a continuous and negatively correlated relationship exists between personality and plasticity. We suggest that each individual's behavior may exhibit a different ratio of these two patterns, which could explain the observed variations in behavior. Some individuals were very willing to change their behaviors (leaning towards plasticity), while others required more time to change (intermediate), and some remained resistant to behavioral changes (leaning towards personality). This proposed relationship between personality and plasticity may provide a framework for understanding the diverse behavioral responses observed in our study and highlights the need for further investigation into the interplay between personality and plasticity.

### Physiological mechanisms for predatory aggressiveness variations

Our transcriptome analysis revealed that the observed personality differences were accompanied by variations in gene expressions across multiple physiological systems, indicating that aggressiveness is a complex personality phenotype modulated by many neurophysiological systems, a scenario that may be applicable to other personality traits as well. We found apparent expression differences in genes associated with neural transmission and signal transduction systems. Most variations were due to relevant genes being considerably upregulated in the HA group. According to the Pace-of-Life Syndrome (POLS) theory, this pattern may reflect that HA individuals, often described as 'big-engine' types with higher metabolic and physiological capacities<sup>2,34,36</sup>, also exhibit enhanced neural signal transduction and transmission activities, because this upregulation could indicate greater brain processing capacity associated with their proactive behavioral tendencies. Additionally, the expression differences in amine systems were consistent with the results of previous mechanistic studies on animal personality, showing that amine systems indeed played a crucial role in shaping personality variation<sup>37,40</sup>. Spiders in the HA group also showed higher expression levels of genes involved in sensory systems compared to those in the LA group. This finding was consistent with the results of previous research on predatory aggression variation in northern spike fish, which also found differences in sensory organs between individuals with different aggressive levels. Sensory systems may be closely associated with predators' aggression behaviors due to their potential direct effect on predatory ability<sup>20</sup>. Notably, a 50% expression difference was observed in genes related to phototransduction between HA and LA individuals, indicating that vision might play a critical role in predatory aggression variation in spiders like *H. holmerae*.

Expression patterns of genes associated with memory, learning, and salivary secretion varied between low and high aggressiveness individuals. Transcriptome analysis showed that genes involved in neurotrophin signaling pathways and long-term potentiation, which are critical for memory and learning<sup>41,42</sup>, were considerably upregulated in the HA group. While predatory aggression had rarely been directly linked to memory and learning in personality studies, previous studies had explored the relationship between aggressive behavior and memory/learning. For example, studies had investigated how winner and loser effects influenced aggression or how spatial memory affected predatory behaviors<sup>43,44</sup>. Although the aggressive behaviors in these studies could not be directly classified as personality, they suggested that memory and learning ability might influence aggression through various mechanisms, thus potentially affecting aggressive personality. The upregulation of genes in memory and learning-related systems of the HA group suggests that HA individuals might have enhanced memory and learning activities in their brain, which could indirectly imply that HA individuals have better memory and learning ability compared to LA individuals. However, since we did not perform transcriptome analyses on individuals without apparent personality (i.e., members of the middle group), it is possible that middle group individuals have even higher memory and learning ability and, consequently, significantly higher expression of related genes compared to HA group individuals. This possibility is especially relevant considering that middle group individuals were the only ones who apparently modified their behaviors when repeatedly interacting with ants, which might potentially indicate their better memory and learning ability.

An interesting finding from our transcriptome analyses is that the expression level of genes associated with salivary secretion differed by more than 40% between the HA and LA individuals. Since we did not remove fangs from the spider specimens used in the analyses, we speculated that these salivary secretion genes were likely involved in the production of spider venom used in subduing prey. A recent review of spider personality studies suggested that venom secretion might be one of the important physiological traits related to personality and could shape the living strategies of spiders, and more proactive individuals might have higher levels of venom production<sup>45</sup>. Consistent with this hypothesis, the pattern that most genes in the salivary secretion system were upregulated in the HA group might suggest that HA individuals have a more active venom production ability. Furthermore, we discovered that the expression patterns of genes associated with circadian rhythm, a physiological system rarely explored in previous personality studies, differed between HA and LA individuals. Circadian rhythm systems are related to the regulation of multiple physiological traits<sup>46–48</sup> and have been found to be positively correlated with metabolic rates in some invertebrates including crickets and fruit flies<sup>49,50</sup>. The upregulation of relevant genes in HA individuals suggests that they might have relatively better circadian rhythmicity and, consequently, higher metabolic rates.

When examining the transcriptome analysis results in the context of POLS theory and energy management models, the consistent pattern of gene upregulation in various physiological systems seems to be congruent with the predictions of these theoretical frameworks. POLS theory and energy management models hypothesized that "big engine" individuals (i.e., HA individuals) followed a higher energy intensity investment strategy characterized by physiological traits such as higher metabolic rates and brain capacities as well as stronger

physical abilities. Consequently, these individuals are expected to exhibit a more agonistic personality to obtain more food intake (i.e., energy-gain behaviors) due to their high energy-demanding molecular and physiological activities. Conversely, “small-engine” individuals (i.e., LA individuals) followed a lower energy intensity investment strategy characterized by lower metabolic rates and brain capacities as well as weaker physical abilities, resulting in a less agonistic personality and reduced food intake due to their low energy-demanding molecular and physiological activities<sup>31–36,51</sup>. Such theoretical frameworks may explain the apparent expression differences in a diverse way of systems observed between HA and LA individuals in our study. Furthermore, it may also account for the upregulation of most genes in HA group individuals compared to LA group individuals.

In this present study, we discovered that the brain tryptophan level of LA group spiders was significantly higher than that of HA group, which is consistent with the correlation between tryptophan deficiency and aggression reported in previous studies<sup>37–39</sup>. Since all spiders used in this study were raised in the laboratory until reaching adulthood and fed with a uniform diet, the observed brain tryptophan variation was unlikely to result from differences in food intake. In mammals, the brain can only utilize tryptophan and its derivatives (such as serotonin) that have been transported across the blood-brain barrier and synthesized within the brain. Furthermore, less than 10% of tryptophan is transported to the brain for utilization, while the remainder is metabolized and used in other parts of the body<sup>32</sup>. Therefore, if such a mechanism does exist in spiders, it is possible that there is only a small proportion of tryptophan being transported into the brain, which will make variations in tryptophan influx a potentially important factor that could affect our results. Currently, the controlling mechanisms of tryptophan influx are not clear. Due to the limited understanding on tryptophan, it is challenging to speculate the mechanisms behind the observed concentration differences and link them to personality variations. While tryptophan is involved in various physiological processes such as neurotransmission, circadian rhythm, sensory perception, and protein synthesis<sup>53–55</sup>, and our transcriptome analyses revealed the upregulation of genes associated with these processes in HA spiders, further research is needed to elucidate the specific mechanisms behind the observed tryptophan concentration differences and their potential connection to aggression and personality traits in spiders.

### Implications and directions for future research

Although the results of our study demonstrated that in spiders with aggressive personalities a great number of genes involved in physiological systems such as signal transmission/transduction, sensory, memory, learning, and circadian rhythm were upregulated, the detailed molecular and physiological mechanisms remain unknown. In future studies, methodologies such as drug manipulation or line breeding should be applied to reveal the detailed mechanisms leading to personality traits. Similarly, although we found indirect evidence from transcriptome analyses that the relevant genes were upregulated so basal metabolic rates might be higher in the HA group individuals, we suggest that future studies directly measure metabolic rates to provide direct evidence for the POLS hypothesis, which can further verify the existence of big-engine and small-engine individuals in the populations. Furthermore, it is still unclear how various selection pressures affect these individuals and generate relevant ecological and evolutionary consequences. Future studies may investigate how the presence of big and small engine individuals affects various population characteristics and community interactions to understand how personality traits are involved in shaping various aspects of ecosystems. Results of our behavioral tests show that not all individuals in the population exhibit apparent personalities. However, in many relevant studies, such an issue was not seriously considered or appropriately controlled. Only when the personality status of an individual is verified could it be used to investigate relevant physiological mechanisms or ecological consequences. Therefore, we recommend that in future studies behavioral tests with a sufficient number of repeated trials should be adopted to verify the tendency of proactive personality and adaptive plasticity in an individual or a population. Without such confirmation, results of subsequent mechanistic or ecological investigations are very likely to be biased.

Sometimes, a number of specific personality traits may express together and this phenomenon is called personality syndrome<sup>56</sup>. Results of our transcriptome analyses demonstrated that genes of numerous physiological systems were simultaneously upregulated in individuals with high aggressive personality. Such findings indicate that there might be other personality traits co-expressed with aggressiveness and form a personality syndrome. Future studies may verify whether there exists a predatory aggressiveness syndrome by identifying other personality traits involved and their association with the observed physiological system variations. For example, according to the energy management models, energy-expenditure and energy-gain behaviors might both be correlated with metabolic rates<sup>51</sup>. These behavioral traits are important components of predation and relevant information about their interaction patterns and underlying mechanisms will greatly improve our understanding of personality syndromes. In addition to the acquisition of energy, nutrient composition, especially amino acids, had been shown to affect spiders' predatory behaviors<sup>57–59</sup> as well as the personality of other animals<sup>60,61</sup>. Therefore, we recommend that future studies examine whether and how amine system-related amino acids affect an individual's personality and plasticity through variations in prey and nutrient intake.

Finally, spiders are the most diverse and abundant invertebrate predators in terrestrial ecosystems. They exhibit great impacts on insect communities and therefore have great potential in biological control<sup>62–64</sup>. There is abundant empirical evidence that spiders could effectively control pest populations in various agricultural systems<sup>65–67</sup>. For example, because of their high abundance in agricultural areas and other artificial environments, *H. holmerae* has great potential in biological control<sup>68,69</sup>. In theory, aggression could affect spiders' impact on insect communities through intra/inter-species interactions<sup>70</sup>. We recommend future research to focus on whether and how spider predatory aggressive personality differences affect arthropod community interactions, and how personalities of individuals in a predator population can be manipulated to enhance their overall biocontrol performance.

## Experimental procedures

### Animals used in the predatory interactions

In this study, garden wolf spiders *Hippasa holmerae* (Araneae, Lycosidae) were used as the experimental animal and all spiders were collected from Taichung Metropolitan Park, Taichung City, Taiwan (120.598254, 24.207399). In order to control age, mating experience, food intake and living environments of spiders to be used in behavioral tests, we only collected juvenile females and kept them in the laboratory until they matured. Each spider was kept individually in a 14 cm × 8 cm × 7.5 cm transparent plastic box with a cotton pellet inside to maintain moisture and to provide water for spiders. Spiders were housed in a controlled laboratory with a 12:12 h light-dark cycle and room temperature of 25 ± 1 °C. We added water to cotton pellet and fed each spider one cockroach (*Blatta lateralis*) with a body length of approximately 0.5 mm once a week.

We used East Indian harvest ants *Carebara diversa* (Hymenoptera, subfamily Myrmicinae) to interact with the garden wolf spiders in the behavioral tests. We frequently found such ants in spiders' habitats, indicating that garden wolf spiders did interact with East Indian harvest ants in the field. The ants were collected by a mini vacuum cleaner (Mini Vacuum Cleaner, Separating Co., China) from the campus of Tunghai University, Taichung, Taiwan (120.595659, 24.180013). Only workers were collected and attention was paid to make sure that all ants used in the behavioral tests were similar in body size. The ants were kept in an artificial ant nest composed of an 8 cm × 8 cm × 8 cm transparent acrylic feeding space and a 10 cm × 10 cm × 5 cm plaster nest. We placed a bottle cap with cotton pellets inside the feeding space and added water twice a week to maintain moisture and provide water. We fed the ants three adult *B. lateralis* cockroaches with a body length of about 25 mm every week. The ants were housed in the same laboratory environment as the spiders. To make sure that the ants were energetic, we collected them one week before each behavioral test and collected hundreds of individuals each time.

### Behavioral test – assessing responses of spiders to ants

In this study, individual spider level of aggressiveness in terms of willingness to attack a highly risky but low-value prey was assessed. Previous studies showed that ants served as a potential threat to spiders and they even affected the evolution of spiders<sup>71,72</sup>. The East Indian harvest ants *C. diversa* are highly aggressive and exhibit collective wandering and hunting behaviors similar to army ants<sup>73</sup>. The body length of a worker ant is about 2.5 to 3.5 mm, which is about half the body length of an adult female *H. holmerae* wolf spider (~ 8 mm). In our behavioral tests, *C. diversa* were used as a highly risky but low-value prey to spiders because all ants could defend themselves well with jaws during the spider attack. In preliminary behavioral tests we found that the bites and venom of wolf spiders were not effective against the ants, and almost all ants retrieved from the behavioral tests could still live normally for more than two weeks. If the spiders want to hunt the ants, they could only drag the ants to their nest with a high risk of getting injured.

Although most wolf spiders are not web weavers, *H. holmerae* is an exception<sup>74</sup>. They will build a low-sticky sheet web with a tunnel, and wait inside the tunnel until prey walks into their sheet web. Because the web is not sticky enough, *H. holmerae* have to engage in hand-to-hand combat with prey and drag them into the tunnel. When *H. holmerae* are confronting *C. diversa*, the latter will fight back aggressively and maintain limited mobility on the sheet web. From the beginning to the end of the behavioral test, the spiders could decide whether to hunt the ants or not. Any time the spiders decided to terminate the hunting, the ants could leave the sheet web in a few minutes. These observations indicate that *C. diversa* is well suited to serve as a highly risky but low-value prey to *H. holmerae*.

### Behavioral test – scoring the level of attacking willingness

To begin the behavioral test, we first opened the lid of the box containing the spiders, then waited for 10 min to allow the spiders to recover from the disturbance of the opening. Then we carefully picked up an ant with a tweezer and placed it on the spider's sheet web. We recorded the predator-prey interaction for 90 s and determined the level of aggressiveness (from 0 to 4 points) according to a scoring scheme (Table 1). If the ant was not successfully hunted by the spider, we would remove the ant to prevent it from hurting or disturbing the spider. From preliminary observations we discovered various behavioral responses of spiders to ants and we scored behavioral scenarios according to spiders' attack willingness. When a spider senses the presence of prey on the web, it usually emerges out of tunnel (defined as "exploring"). After detecting the presence of an ant on the web, the spider might either move back to the tunnel without any attempt to attack (defined as "giving up"), bite the ant one or two times (defined as "biting"), bite the ant more than three times (defined as "multiple biting"), or try to drag the ant to its tunnel (defined as "dragging"). If, after emerging from the tunnel, the spider gave up attack and went back to the tunnel, we scored such a behavioral scenario as 0 points. After biting the ant, sometimes the spider might simply stand beside the prey and watch it for a while (defined as "watching"). When the spider exhibited watching behavior, it would be given either 2 or 3 points depending on the type of prior biting behavior (biting vs. multiple biting). Finally, because dragging an energetic and struggling ant is associated with a high risk of injury compared to other hunting behaviors, it represents a higher willingness to attack than biting. Therefore, we designated "dragging" as the highest level of aggressiveness and scored such behavior 4 points, no matter what type of biting exhibited by spider. (See video clips of 0, 1, 2 and 4-point events in Supplementary Materials)

We followed the following principles during the behavioral tests to ensure the quality of the results. Firstly, we stopped feeding the spiders one week before the behavioral test to make sure that they had hunting willingness. Secondly, to ensure that the test results were not affected by sex, age or mating experience, we only used female spiders that grew in the laboratory from juveniles to adults. Thirdly, considering the circadian rhythm of spiders, we only performed the behavioral tests between 14:00 and 17:00. Fourthly, if the spider was bitten by the ant, or the spider did not show exploring behavior within 90 s, the trial was terminated and test would be performed

again after two days. Fifthly, if the ant escaped from the web within 90 s rendering the spider having not enough time to react, we would remove the ant and repeat the trial after one hour. Finally, since personality traits needed to show repeatability, there were five repeated trials with two-day intervals in each behavioral test, and a spider's level of aggressiveness was determined by the average score of five trials.

### Grouping of spiders according to attack willingness

For further analyses, we divided spiders into three different groups according to their average behavioral test scores. Spiders with a mean score smaller than 1 point were designated as the LA (Low Aggressiveness) group; those with a mean score greater than 3 points were designated as the HA (High Aggressiveness) group; and the rest of the individuals were designated as the middle group. Although a more strict grouping pattern - such as using 0.5 and 3.5 points as thresholds, could improve the AIC values of models, the sample sizes in HA and LA groups would be too small for subsequent analyses. Therefore, 1.0 and 3.0 points were used as thresholds for grouping spiders to ensure sufficient sample size for effective statistical treatments.

### Modeling personality and plasticity with GLMM and GLM

We used a model-building approach based on Generalized Linear Mixed Models (GLMM) and Generalized Linear Models (GLM) to best describe our behavioral data and to achieve two objectives. Firstly, we elucidated the relationships between plasticity and personality by testing whether there were apparent personality and plasticity effects within each group. Secondly, we examined whether there was a personality difference between groups if there was apparent personality present in each group.

We employed an approach similar to the methodology described by O'Dea et al. (2022)<sup>2</sup>, which used slope to represent plasticity and both repeatability and consistency to indicate personality in a linear model. Here, we used ID numbers as the random factor to assess the personality effect and the trial numbers as the fixed factor to assess the plasticity effect. Because, when individuals of strong personality exhibit a highly repeatable and consistent behavior, the random factor ID number can explain more variations in behaviors. In contrast, if individuals' behaviors are easily influenced by the environmental effects and show plasticity, the fixed factor, trial numbers, can explain more variations in behaviors.

We constructed six models with Poisson distribution to appropriately analyze our ordinal aggression score data (0–4 points). These models were categorized into three types based on different grouping strategies: “mixture,” “middle,” and “extreme.” The model “mixture” included all individuals; the model “extreme” included only individuals from the HA and LA groups; and the model “middle” included only individuals from the middle group. For each type, we built two models: one treating trial number as a categorical variable (comparing each trial to baseline trial 1), and the other treating trial number as a numerical variable (to assess linear trends from trial 1 to 5).

Since age, sex, size, environments and food were all under good control, we did not include these factors in the analyses. Also, although we initially attempted to construct more complex models that included not only personality (ID) and plasticity (trial number) effects, but also their interaction (ID × trial number), these models failed to converge and showed increased AIC values, suggesting that simpler models without the interaction term could best describe our data (See Supplementary Information 4 for the failed converge model code). All GLM and GLMM analyses were performed in R version 4.4.2 using the *lme4* package<sup>75</sup>.

### Transcriptome analysis - RNA extraction

All individuals used in this part of the study were sacrificed within 1 month after the behavioral tests. To minimize animal suffering during sacrifice, spiders were first anesthetized with CO<sub>2</sub>. The dissection was performed on an ice plate to prevent recovery from anesthesia and was completed within one minute for each spider. The prosoma was immediately transferred to liquid nitrogen and subsequently stored at –80 °C for further analyses. We used three extremely aggressive individuals with a mean score higher than 3.5 points from HA group and three extremely non-aggressive individuals with a mean score lower than 0.5 point for transcriptome analyses. We did not use individuals from the middle group because they seemed less aligned with personality traits in their predatory behavior. During extraction, we first transferred each sample into a 1.5 ml Eppendorf with 0.5 ml trizol, and ground it thoroughly with a polypropylene grinding rod. Then RNA extraction was performed according to the protocols on the website of Thermo (<https://reurl.cc/gQo4eb>), and Nanodrop was used to assess the quality (value of A260/280) and concentration of the RNA.

### Transcriptome analysis - library construction

Before library construction, we performed DNase treatment using the Invitrogen TURBO DNA-free Kit from Thermo (<https://reurl.cc/MXo4Wp>). Then, we constructed libraries using Universal Plus mRNA-Seq with NuQuant (<https://reurl.cc/WqoRVL>, TECAN). After library construction, Qubit<sup>®</sup> 2.0 Fluorometer with dsDNA High Sensitivity Kit and DNA 1000 assay of Agilent 2100 Bioanalyzer were used to assess the quality and concentration of each library. When RNA quality was satisfactory, 2 × 151 bp paired-end sequencing was performed using NovaSeq 6000 sequencing system. After sequencing, QC test and trimming were conducted using QV ≥ 20 by CLC Genomics Workbench v10 to prevent low-quality sequences from affecting further analyses.

### Transcriptome analysis – annotation and quantification

Since currently there is no reference sequence data of *H. holmerae*, we performed the de-novo assembly method using Trinity<sup>76</sup> and Strand-specific RNA-Seq read orientation, followed by Contig clustering using CD-HIT<sup>77,78</sup>. Finally, BlastX analysis was performed to annotate each contig by DIAMOND<sup>79</sup> with the nr database. Annotated sequences' information was all uploaded to the NCBI database. To find out genes exhibiting

significant differences between the HA and LA groups, RNA-Seq analysis of CLC Genomics Workbench v10 was used to calculate the read numbers of each contig. Since longer sequences were associated with higher read numbers, Fragments Per Kilobase of exon model per Million mapped fragments (FPKM) was used to standardize the expression level of each gene<sup>80</sup>. (see Supplementary Information 1 for detailed methods) ( $FPKM = \frac{\text{total exon fragments}}{\text{mapped fragments(millions)} \times \text{exonlength(KB)}}$ ). All annotated sequences were uploaded to NCBI database. (see Supplementary Information 2 for annotated sequences' information).

### Transcriptome analysis – comparison between groups

After annotation and quantification, those genes with more than 2X expression difference between groups were verified by DESeq2 package in R<sup>81</sup> and were designated as Differential Expression Genes (DEGs), and were illustrated in a volcano plot (see Figure S1 of Supplementary Information 1). These analyses would help verify whether categorizing spiders into the HA and LA groups could be supported by the results of transcriptome analyses. To understand gene differences systematically, we performed pathway analysis by using KEGG Automatic Annotation Server (KAAS) to make KEGG Orthology (KO) ID prediction<sup>82–86</sup>. We also calculated percent differences to find out physiological systems exhibiting large gene expression differences between the HA and LA groups. (see Supplementary Information 1 for detailed methods, and Supplementary Information 6 for sequences' information with KO ID prediction).

### HPLC analysis – chemical extraction

To further understand the differences in amine systems identified from transcriptome analyses, we used HPLC-MS/MS to estimate the concentrations of various amines and related amino acids. Six individuals from HA group with a mean score of 3.267 points and another six individuals from LA group with a mean score of 0.533 point were used to perform HPLC analyses. Samples were placed in a 1.5 ml Eppendorf with 0.2 ml 0.3% formic acid in ddH<sub>2</sub>O and were ground with a polypropylene grinding rod followed by 60 s of sonication. Then the samples were centrifuged for 10 min (15000 rpm at 4°C), and the supernatant was centrifuged again with a 10 K molecular sieve for 20 min (14000xg at 4°C). Samples were stored at –20°C for further analysis.

### HPLC analysis - HPLC-MS/MS

We used an Ultra-High Performance Liquid Chromatography (UHPLC) with a Quadrupole-Time of Flight Mass Spectrometer (Q-ToF), and an Acclaim RSLC120 C18 tube (150 mm × 2.1 mm). All analyses were performed at 30°C and the volume of each sample was 20 µl. For UHPLC analyses, 0.3% formic acid in ddH<sub>2</sub>O (liquid A) and 0.3% formic acid in acetonitrile (liquid B) were used, and the flow rate was set at 0.075 ml/min. Transitions of liquid concentrations were as follows: 100% liquid A for 4 min, liquid A decreased to 90% in 5 min, liquid A decreased to 0% in 6 min, liquid A kept at 0% for 7 min, and liquid A increased to 100% in 13 min. We used a mass spectrometer to quantify and verify the identity of chemicals. Electrospray ionization (ESI) parameters were set as follows: flow rate 200 µl/min, electric current 500nA, voltage 3500 V, flow rate of gas Nitrogen 9 L/min, temperature 200°C, and length 0.25 mm. During the first MS, we identified chemical identity by flow rate and calculated concentration by area with positive ion mode. During the second MS, we identified chemicals detected in the first MS phase more precisely with positive ion mode and multi-reaction monitoring mode (MRM).

After verifying the identity of the chemicals and quantifying their concentrations, Student t-tests were performed to determine whether there were significant differences in concentration of various chemicals between the HA and LA groups. Before the t-tests, we first performed Shapiro-Wilk tests to make sure that relevant data followed normal distribution (Histamine: LA  $p = 0.987$ , HA  $p = 0.751$ ; Acetylcholine: LA  $p = 0.07$ , HA  $p = 0.491$ ; Glutamate: LA  $p = 0.266$ , HA  $p = 0.646$ ; Tryptophan: LA  $p = 0.433$ , HA  $p = 0.797$ ). We also performed F-tests to determine the appropriate t-tests to be used (Histamine:  $p = 0.01$ ; Acetylcholine:  $p = 0.027$ ; Glutamate:  $p = 0.885$ ; Tryptophan:  $p < 0.001$ ). Standard t-tests were used when variance was equal ( $p > 0.05$  in F-test) while Welch's t-tests were used when variance was not equal ( $p < 0.05$  in F-test). All statistical analyses were performed in R version 4.2.2<sup>87</sup>. (see HPLC results and its code for analysis in Supplementary Information 5).

### Data availability

We provide all transcriptome sequences' NCBI annotated results and the expression level comparison results in xlsx format in Supplementary Information 2. The behavioral data and the R code for building GLMM and GLM models are provided in Supplementary Information 3 and 4. Additionally, the results of the HPLC-MS/MS analysis and the corresponding analysis code are provided in Supplementary Information 5. The results of the behavioral tests organized in txt format and video clips of 0, 1, 2, and 4-point events are provided in the Supplementary Materials, along with video legends in Supplementary Information 1. Regarding the transcriptome analysis data, although we cannot provide the analysis code, we provide detailed methods in Supplementary Information 1. Detailed KEGG pathway analysis results are provided in Supplementary Information 6 in xlsx format. The transcriptome sequence data are also deposited in the NCBI Sequence Read Archive (SRA) under accession number SRR31147554 in FASTQ format. (<https://www.ncbi.nlm.nih.gov/sra?term=SRR31147554>).

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### References

1. Kaiser, M. I. & Müller, C. What is an animal personality? *Biology Philos.* **36**, 1 (2021).

2. O’Dea, R. E., Noble, D. W. A. & Nakagawa, S. Unifying individual differences in personality, predictability and plasticity: a practical guide. *Methods Ecol. Evol.* **13**, 278–293 (2022).
3. de Azevedo, C. S. & Young, R. J. Animal personality and conservation: basics for inspiring new research. *Animals*, **11**, 1019. (2021).
4. Liedtke, J., Redekop, D., Schneider, J. M. & Schuett, W. Early environmental conditions shape personality types in a jumping spider. *Front. Ecol. Evol.* **3**, 134 (2015).
5. Liebgold, E. B. The influence of social environment: behavior of unrelated adults affects future juvenile behaviors. *Ethology* **120**, 388–399 (2014).
6. Chang, C. C. et al. Female spider aggression is associated with genetic underpinnings of the nervous system and immune response to pathogens. *Mol. Ecol.* **29**, 2626–2638 (2020).
7. Dochtermann, N. A., Schwab, T. & Sih, A. The contribution of additive genetic variation to personality variation: heritability of personality. *Proc. Royal Soc. B: Biol. Sci.* **282**, 20142201 (2015).
8. Dingemanse, N. J. & Wolf, M. Recent models for adaptive personality differences: a review. *Philosophical Trans. Royal Soc. B: Biol. Sci.* **365**, 3947–3958 (2010).
9. Dubnau, D. & Losick, R. Bistability in bacteria. *Mol. Microbiol.* **61**, 564–572 (2006).
10. Nettle, D. The evolution of personality variation in humans and other animals. *Am. Psychol.* **61**, 622–631 (2006).
11. Jones, T. C. et al. Octopamine and serotonin have opposite effects on antipredator behavior in the orb-weaving spider, *Larinioides cornutus*. *J. Comp. Physiol. A.* **197**, 819–825 (2011).
12. Johnson, O., Becnel, J. & Nichols, C. D. Serotonin 5-HT (2) and 5-HT (1A)-like receptors differentially modulate aggressive behaviors in *Drosophila melanogaster*. *Neuroscience* **158**, 1292–1300 (2009).
13. Buchanan, S., Kain, J. & de Bivort, B. Neuronal control of locomotor handedness in *Drosophila*. *Proc. Natl. Acad. Sci. U.S.A.* **112**, 6700–6705 (2015).
14. Agrawal, A. A. Phenotypic plasticity in the interactions and evolution of species. *Science* **294**, 321–326 (2001).
15. Berg, M. P. & Ellers, J. Trait plasticity in species interactions: a driving force of community dynamics. *Evol. Ecol.* **24**, 617–629 (2010).
16. Miner, B. G., Sultan, S. E., Morgan, S. G., Padilla, D. K. & Relyea, R. A. Ecological consequences of phenotypic plasticity. *Trends Ecol. Evol.* **20**, 685–692 (2005).
17. Cornwell, T. O., McCarthy, I. D., Snyder, C. R. A. & Biro, P. A. The influence of environmental gradients on individual behaviour: individual plasticity is consistent across risk and temperature gradients. *J. Anim. Ecol.* **88**, 511–520 (2019).
18. Dubois, F. Why are some personalities less plastic? *Proc. Royal Soc. B: Biol. Sci.* **286**, 20191323 (2019).
19. Hall, M. L., Parson, T., Riebel, K. & Mulder, R. A. Personality, plasticity, and resource defense. *Behav. Ecol.* **28**, 138–144 (2017).
20. McGhee, K. E., Pintor, L. M. & Bell, A. M. Reciprocal behavioral plasticity and behavioral types during predator-prey interactions. *Am. Nat.* **182**, 704–717 (2013).
21. Freudenberg, F., Carreño Gutierrez, H., Post, A. M., Reif, A. & Norton, W. H. Aggression in non-human vertebrates: genetic mechanisms and molecular pathways. *Am. J. Med. Genet. Part. B: Neuropsychiatric Genet.* **171**, 603–640 (2016).
22. Iba, M., Nagao, T. & Urano, A. Effects of population density on growth, behavior and levels of biogenic amines in the cricket, *Gryllus bimaculatus*. *Zoolog. Sci.* **12**, 695–702 (1995).
23. Schuett, W., Tregenza, T. & Dall, S. R. X. Sexual selection and animal personality. *Biology* **85**, 217–246 (2010).
24. Dingemanse, N. & Réale, D. Natural selection and animal personality. *Behaviour* **142**, 1159–1184 (2005).
25. McNamara, J. M., Stephens, P. A., Dall, S. R. X. & Houston, A. I. Evolution of trust and trustworthiness: social awareness favours personality differences. *Proceedings of the Royal Society B: Biological Sciences*, 276, 605–613. (2009).
26. Lichtenstein, J. L. L., Rice, H. K. & Pruitt, J. N. Personality variation in two predator species does not impact prey species survival or plant damage in staged mesocosms. *Behav. Ecol. Sociobiol.* **72**, 70 (2018).
27. Toscano, B. J., Lichtenstein, J. L. L. & Costa-Pereira, R. Intraspecific behavioral variation mediates insect prey survival via direct and indirect effects. *Diversity* **12**, 152 (2020).
28. Carere, C. & Maestripieri, D. *Animal personalities: Behavior, physiology, and evolution*. University of Chicago Press. ISBN: 978-0226081399. (2013).
29. Réale, D. et al. Personality and the emergence of the pace-of-life syndrome concept at the population level. *Philosophical Trans. Royal Soc. B: Biol. Sci.* **365**, 4051–4063 (2010).
30. Pruitt, J. & Riechert, S. The ecological consequences of temperament in spiders. *Curr. Zool.* **58**, 589–596 (2012).
31. Réale, D., Dingemanse, N. J., Kazem, A. J. N. & Wright, J. Evolutionary and ecological approaches to the study of personality. *Philosophical Trans. Royal Soc. B: Biol. Sci.* **365**, 3937–3946 (2010).
32. Dammhahn, M., Dingemanse, N. J., Niemelä, P. T. & Réale, D. Pace-of-life syndromes: a framework for the adaptive integration of behaviour, physiology and life history. *Behav. Ecol. Sociobiol.* **72**, 1–8 (2018).
33. Le Galliard, J. F., Paquet, M., Cisel, M. & Montes-Poloni, L. Personality and the pace-of-life syndrome: variation and selection on exploration, metabolism and locomotor performances. *Funct. Ecol.* **27**, 136–144 (2012).
34. Mathot, K. J. & Frankenhuis, W. E. Models of pace-of-life syndromes (POLS): a systematic review. *Behav. Ecol. Sociobiol.* **72**, 41 (2018).
35. Niemelä, P. T., Dingemanse, N. J., Alioravainen, N., Vainikka, A. & Kortet, R. Personality pace-of-life hypothesis: testing genetic associations among personality and life history. *Behav. Ecol.* **24**, 935–941 (2013).
36. Vasilieva, N. A. Pace-of-life syndrome (POLS): evolution of the concept. *Biology Bull. Russian Acad. Sci.* **49**, 750–762 (2022).
37. Rádai, Z. et al. State and physiology behind personality in arthropods: a review. *Behav. Ecol. Sociobiol.* **76**, 150 (2022).
38. Bacqué-Cazenave, J. et al. Serotonin in animal cognition and behavior. *Int. J. Mol. Sci.* **21**, 1649 (2020).
39. Kikuchi, A. M., Tanabe, A. & Iwahori, Y. A systematic review of the effect of L-tryptophan supplementation on mood and emotional functioning. *J. Diet. Supplements.* **18**, 316–333 (2021).
40. Nelson, R. J. & Trainor, B. C. Neural mechanisms of aggression. *Nat. Rev. Neurosci.* **8**, 536 (2007).
41. Lynch, M. A. Long-term potentiation and memory. *Physiol. Rev.* **84**, 87–136 (2004).
42. Miranda, M., Morici, J. F., Zannoni, M. B. & Bekinschtein, P. Brain-derived neurotrophic factor: a key molecule for memory in the healthy and the pathological brain. *Front. Cell. Neurosci.* **13**, 363 (2019).
43. Trannoy, S. & Kravitz, E. A. Learning and memory during aggression in *Drosophila*: handling affects aggression and the formation of a loser effect. *J. Nat. Sci.* **1**, 56 (2015).
44. Gurarie, E. et al. Spatial memory drives foraging strategies of wolves, but in highly individual ways. *Front. Ecol. Evol.* **10**, 768478 (2022).
45. Hernández Duran, L., Wilson, D. T., Briffa, M. & Rymer, T. L. Beyond spider personality: the relationships between behavioral, physiological, and environmental factors. *Ecol. Evol.* **11**, 2974–2989 (2021).
46. Sen, A. & Hoffmann, H. M. Role of core circadian clock genes in hormone release and target tissue sensitivity in the reproductive axis. *Mol. Cell. Endocrinol.* **501**, 110655 (2020).
47. Xu, K., DiAngelo, J. R., Hughes, M. E., Hogenesch, J. B. & Sehgal, A. The circadian clock interacts with metabolic physiology to influence reproductive fitness. *Cell Metabol.* **13**, 639–654 (2011).
48. Yeung, J. & Naef, F. Rhythms of the genome: circadian dynamics from chromatin topology, tissue-specific gene expression, to behavior. *Trends Genet.* **34**, 915–926 (2018).
49. Giebultowicz, J. M. Circadian regulation of metabolism and healthspan in *Drosophila*. *Free Radic. Biol. Med.* **119**, 62–68 (2018).

50. Stahlschmidt, Z. R. Flight capacity drives circadian patterns of metabolic rate and alters resource dynamics. *J. Experimental Zool. Part. A: Ecol. Integr. Physiol.* **337**, 666–674 (2022).
51. Mathot, K. J. & Dingemanse, N. J. Energetics and behavior: unrequited needs and new directions. *Trends Ecol. Evol.* **30**, 199–206 (2015).
52. Roth, W., Zadeh, K., Vekariya, R., Ge, Y. & Mohamadzadeh, M. Tryptophan metabolism and gut-brain homeostasis. *Int. J. Mol. Sci.* **22**, 2973 (2021).
53. Höglund, E., Øverli, Ø. & Winberg, S. Tryptophan metabolic pathways and brain serotonergic activity: a comparative review. *Front. Endocrinol.* **10**, 158 (2019).
54. Kousar, S., Anjuma, S., Jaleel, F., Khan, J. & Naseema, S. Biomedical significance of tryptamine: a review. *J. Pharmacovigil.* **5**, 1–6 (2017).
55. Schwarcz, R. & Stone, T. W. The kynurenine pathway and the brain: challenges, controversies and promises. *Neuropharmacology* **112**, 237–247 (2017).
56. Sih, A., Bell, A. M., Johnson, J. C. & Ziemba, R. E. Behavioral syndromes: an integrative overview. *Q. Rev. Biol.* **79**, 241–277 (2004).
57. Greenstone, M. H. Spider feeding behaviour optimises dietary essential amino acid composition. *Nature* **282**, 501–503 (1979).
58. Price, J. B. Exploring the relationship between behaviour and neurochemistry in the polyphenic spider, *Anelosimus studiosus* (Araneae: Theridiidae). PhD Thesis. East Tennessee State University. (2016).
59. Walker, S. E., Marshall, S. D. & Rypstra, A. L. The effect of feeding history on retreat construction in the Wolf spider *Hogna helluo* (Araneae: Lycosidae). *J. Arachnology.* **27**, 689–691 (1999).
60. Ashley-Ross, M. A. & McMahon, T. A. Tryptophan depletion alters the decision-making of rats on a rodent gambling task: reversal of effects with diazepam. *Behav. Brain. Res.* **223**, 61–69 (2011).
61. Harrison, F. & Hall, L. A resource-mediated trade-off between aggression and exploration in snails. *Anim. Behav.* **79**, 119–125 (2010).
62. Benamú, M. A., Lacava, M., García, L. F., Santana, M. & Viera, C. Spiders associated with agroecosystems: roles and perspectives. *Behaviour and Ecology of Spiders*, 275–302. Springer. (2017).
63. Nyffeler, M. & Birkhofer, K. An estimated 400–800 million tons of prey are annually killed by the global spider community. *Sci. Nat.* **104**, 30 (2017).
64. Stratton, G. E. Review of spiders in ecological webs by D. H. *Wise J. Arachnology.* **23**, 48–50 (1995).
65. García, L. F. et al. Experimental assessment of trophic ecology in a generalist spider predator: implications for biocontrol in Uruguayan crops. *J. Appl. Entomol.* **145**, 82–91 (2021).
66. Michalko, R., Pekár, S. & Entling, M. H. Global patterns in the biocontrol efficacy of spiders: A meta-analysis. *Glob. Ecol. Biogeogr.* **28**, 1366–1378 (2019).
67. Rendon, D. The role of wolf spiders (Araneae: Lycosidae) on the biological control of the bollworm *Helicoverpa spp.* (Lepidoptera: Noctuidae) in cotton crops. PhD Thesis. Macquarie University. (2016).
68. Chandra, U., Singh, I. B. & Singh, H. M. Studies on population dynamics of spider in rice crop regarding biocontrol. *Int. J. Curr. Microbiol. Appl. Sci.* **4**, 116–124 (2017).
69. Yadav, A., Chaubey, S. N. & Beg, M. A. *Hippasa holmerae* Thorell (garden Wolf spider) as biocontrol agent for insect pests of crop fields collected from Azamgarh and Mau districts (U.P.) India. *J. Experimental Zool. India.* **15**, 495–498 (2012).
70. Chang, C. C., Teo, H. Y., Norma-Rashid, Y. & Li, D. Predator personality and prey behavioural predictability jointly determine foraging performance. *Sci. Rep.* **7**, 40734 (2017).
71. Wright, C. M., Keiser, C. N. & Pruitt, J. N. Colony personality composition alters colony-level plasticity and magnitude of defensive behaviour in a social spider. *Anim. Behav.* **115**, 175–183 (2016).
72. Zhang, S. et al. A novel property of spider silk: chemical defence against ants. *Proceedings of the Royal Society B: Biological Sciences*, 279, 1824–1830. (2011).
73. Moffett, M. W. Marauder ants (*Carebara* in Part). In (ed Starr, C.) *Encyclopedia of Social Insects*, 1–4. Springer. (2019).
74. Foelix, R. F. *Biology of Spiders*. 3rd Edition, Oxford University Press, Oxford. (2011).
75. Bates, D., Mächler, M., Bolker, B. & Walker, S. Package lme4: linear mixed-effects models using eigen and S4. R package version, 67. (2014).
76. Grabherr, M. G. et al. Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nat. Biotechnol.* **29**, 644–652 (2011).
77. Fu, L. M., Niu, B. F., Zhu, Z. W., Wu, S. T. & Li, W. Z. CD-HIT: accelerated for clustering the next generation sequencing data. *Bioinformatics* **28**, 3150–3152 (2012).
78. Li, W. Z. & Godzik, A. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. *Bioinformatics* **22**, 1658–1659 (2006).
79. Buchfink, B., Xie, C. & Huson, D. H. Fast and sensitive protein alignment using DIAMOND. *Nat. Methods.* **12**, 59–60 (2015).
80. Mortazavi, A., Williams, B. A., McCue, K., Schaeffer, L. & Wold, B. Mapping and quantifying mammalian transcriptomes by RNA-Seq. *Nat. Methods.* **5**, 621–628 (2008).
81. Love, M. I., Huber, W. & Anders, S. Moderated Estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* **15**, 550 (2014).
82. Moriya, Y., Itoh, M., Okuda, S., Yoshizawa, A. & Kanehisa, M. KAAS: an automatic genome annotation and pathway reconstruction server. *Nucleic Acids Res.* **35**, 182–185 (2007).
83. Storey, J. D., Bass, A. J., Dabney, A., Robinson, D. & Warnes, G. Qvalue: Q-value Estimation for false discovery rate control. *R Package Version* (2015). 2.14.0.
84. Kanehisa, M., Furumichi, M., Sato, Y., Matsuura, Y. & Ishiguro-Watanabe, M. KEGG: biological systems database as a model of the real world. *Nucleic Acids Res.* **53** (D1), D672–D677 (2025).
85. Kanehisa, M. Toward Understanding the origin and evolution of cellular organisms. *Protein Science: Publication Protein Soc.* **28** (11), 1947–1951 (2019).
86. Kanehisa, M. & Goto, S. KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Res.* **28** (1), 27–30 (2000).
87. R Core Team. *R: A Language and Environment for Statistical Computing* (R Foundation for Statistical Computing, 2022).

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## Author contributions

Y.C.L., Y.C.Y., and I.M.T. conceptualized this study. Y.C.L. conducted the research. Y.H.H. assisted in the design and execution of the HPLC experiments. Y.C.L. and I.M.T. wrote the manuscript.

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## Declarations

### Competing interests

The authors declare no competing interests.

### Additional information

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