

Potential investment tradeoff between offspring production and functional recovery promoted by larval cannibalism in *Coccinella septempunctata* (Coleoptera: coccinellidae)

Pengxiang Wu,^{a,b} Baoxu Ma,^{a,b} Haoyong Ouyang,^{a,b} Jing Xu^a and Runzhi Zhang^{a*}

ABSTRACT

BACKGROUND: Since larval cannibalism is frequently observed in intensive rearing systems, the regeneration of lost legs is common for the beneficial species *Coccinella septempunctata* (Coccinella: Coccinellidae) to adapt to the competitive environment, but whether functional recovery occurs in the leg-regenerated coccinellids remains unknown. To evaluate the functional recovery of regenerated right foreleg after being damaged, the behaviors of leg-regenerated ladybugs containing predation, attachment, intraspecific competition, prey preference and fecundity were studied in the laboratory.

RESULTS: The prey consumption and searching rate of leg-regenerated ladybugs decreased, and their handling time extended. A significantly reduced attachment coefficient was detected in leg-regenerated coccinellids. Because of the competitive inferiority, leg-regenerated ladybugs were greatly hampered in competition with normal opponents, and this inferiority led to a switch of prey preference from big-sized adults to small-sized first–second instar nymphs of *Paratrioza sinica*. However, although reduced functional abilities were examined, the leg-regenerated paternity had a higher reproductive output compared to the normal paternity.

CONCLUSION: Leg-regenerated ladybugs caused by cannibalism may make an investment tradeoff between egg fecundity and functional recovery. Thus, larval cannibalism potentially improves the offspring production of the biological control agent in complex environments.

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Keywords: ladybug; leg regeneration; functional behavior; biological control agent

1 INTRODUCTION

Cannibalism is the act of one individual consuming all or part of conspecific competitors as food, which causes physical damage. Predatory coccinellids tend to cannibalize, and the cannibalism events reach occasionally up to 27.1%, the behavior impacts population stability of the beneficial species from a biological control perspective.¹ Superior coccinellids can eliminate intraspecific competitors, but the risk of injury to the larval cannibals is a remarkable cost of cannibalism.² Thus, losing a leg is common for coccinellid larvae, especially under intensive rearing conditions. Regeneration is a capacity of organisms to protect themselves by repairing the lost tissues or organs.^{3,4} *Coccinella septempunctata* having a leg-regeneration capacity has been identified.⁵ Even though lost legs caused by cannibalism could be regenerated morphologically, various functional behaviors are potentially impaired containing predation,⁶ locomotion,⁷ competition,^{8–10} and mating.^{11–13}

The foraging ability is impacted primarily although legs are regenerated under cannibalism situations. Animals invest more nutrients in tissue regeneration and neglect the body growth.¹⁴ Thus, leg-regenerated individuals are likely to avoid further

capture attempts, triggering a decreased foraging efficiency.^{15,16} Impaired functions of regenerated legs may cause an alternative selection between safe habitats with scarce prey and risk habitats with easy predation, and this choice could affect body sizes or conditions of the predators over evolutionary time.¹⁷ Legs are particularly important to coccinellids as they capture by using their legs for sensory detection and physical restraint. Sensory hairs on regenerated legs are occasionally malformed, which may impact the searching rate,¹⁸ so newly regenerated legs sometimes are less or even not functional in foraging behaviors.¹⁹

Various attachment systems on the feet help animals move freely on smooth surfaces.^{20–22} One of them is the hairy system enhancing friction of the adhesive pads across insect groups.^{23–26}

* Correspondence to: R Zhang, Department of Entomology, Institute of Zoology, Chinese Academy of Sciences, #1-5 Beichen West Rd., Chaoyang, Beijing 100101, China. E-mail: zhangrz@ioz.ac.cn

a State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing, China

b University of Chinese Academy of Sciences, Beijing, China

Some adhesive pads are reported to have an ability to secrete fluids contributing to attachment.^{27,28} Adhesive pads of beetles releasing secretions are identified as a wet attachment system,^{29–31} and this hairy system helps them attach to various natural substrates.^{32,33} After cannibalism occurs, the attachment system of the regenerated legs may be affected owing to the hairy system being damaged.

Interactions in predator–prey systems are complex, especially in the environment where competition is intensive.³⁴ Among them is intraspecific competition involving conspecific predators,³⁵ and it has been established in predaceous coccinellids.³⁶ Limb-regenerated individuals with reduced competitive abilities are greatly hampered in competition with normal opponents.^{37–39} Besides, leg-regenerated predators have a tendency to hunt smaller prey,⁴⁰ so cannibalism may impact the prey preference of leg-regenerated coccinellids.

Parental generations with leg regeneration produce more viable offspring in *Menochilus sexmaculatus*, suggesting the possible existence of reproductive investment.⁴¹ As the behavioral function can not be fixed completely after larval cannibalism, leg-regenerated *Harmonia axyridis* tends to make an investment in offspring fitness.⁴² Paternal effects are also found in *Cheilomenes sexmaculata* and the regenerated paternity can obviously increase egg fertility.⁴³

In the present study, natural cannibalism failed to keep injury sites consistent. Since both artificial amputation and natural cannibalism result in a similar outcome which is that tissues of legs are physically damaged, artificial amputation keeping the same ablation site was performed to imitate larval cannibalism, then the various functional behaviors of leg-regenerated *Coccinella septempunctata* were tested. Our goal in this study was to evaluate the level of functional recovery of leg-regenerated ladybugs by detecting the behaviors containing predation, attachment, intraspecific competition, prey preference and fecundity.

2 MATERIALS AND METHODS

2.1 Insects

The 4th-instar larvae of seven-spotted ladybugs *Coccinella septempunctata* and their prey *Paratrioza sinica* Yang & Li were taken from Dadi eco-cultivation bases of *Lycium barbarum* L., Zhongning County, Ningxia Province, China. Larvae or adults of *Coccinella septempunctata* were held in plastic containers (16 cm × 22 cm × 8 cm), reared with fresh *Paratrioza sinica* under the condition of a 16 h:8 h light:dark (L:D) at 28–30 °C and 40–60% relative humidity (RH). The right foreleg (dorsal view) of the 4th-instar larvae of *Coccinella septempunctata* was damaged at the base of the coxa to imitate larval cannibalism. After anesthesia, the larvae were placed on double-sided tape, and the legs were amputated using a pair of micro-scissors. Then the leg-damaged larvae were held and fed in the same conditions as before. The newly emerging adults (<12 h) with leg regeneration were served as treated subjects, and all emerging adults was starved for 24 h before predation experiments.

2.2 Foraging ability

The experiment was performed to calculate the functional response of *Coccinella septempunctata* adults with leg regeneration on *Paratrioza sinica*. Adults of *Paratrioza sinica* at each prey density were provided to a leg-regenerated ladybug in a plastic container, prey densities tested were 100, 150, 200, 250 or 300.

The number of *Paratrioza sinica* consumed after 24 h was counted to evaluate the searching rate and the handling time. Normal ladybugs were served as control. Each treatment was replicated 30 times (15♀:15♂) simultaneously.

2.3 Attachment ability

To analyze the effect of cannibalism on the attachment ability, a small piece of sponge used as a weight was connected to a leg-regenerated adult of *Coccinella septempunctata* via string. The ladybug was placed on a glass plate in an horizontal orientation, then the plate was turned upside down. Water was slowly injected into the sponge until the *Coccinella septempunctata* fell off the plate. The body mass and the critical hanging weight leading to the beetles falling off were detected by analytical balance, and the attachment coefficient (ratio of the critical hanging weight to the body mass) was calculated. Normal ladybugs were used as control. Each treatment was replicated 40 times (20♀:20♂) simultaneously.

2.4 Competitive ability

To evaluate the competitive ability of leg-regenerated *Coccinella septempunctata* in competition with the normal individuals, the coefficients of mutual interference between leg-regenerated and normal adults were examined. Same-sex, four day-old *Coccinella septempunctata* adults were paired (one regenerated adult and one normal adult) in a plastic container, the prey densities tested were 60, 120, 180, 240 and 300 *Paratrioza sinica* adults for one, two, three, four and five pairs of predators, respectively (the prey/predator ratio was kept at 60 for each pair of coccinellids). Same five density gradients of *Paratrioza sinica* adults for one, two, three, four and five pairs of normal ladybugs were served as control. Consumption rates of both leg-regenerated and normal ladybugs were calculated, and then the coefficients of mutual interference were estimated. Each treatment was replicated ten times (5♀:5♂) simultaneously.

Consumption of regenerated ladybug =

$$\left(\text{Consumption of regenerated} - \text{normal couple} \right) - \left[\left(\text{Consumption of normal} - \text{normal couple} \right) / 2 \right].$$

2.5 Prey preference

In order to explore the prey preference of leg-regenerated *Coccinella septempunctata* attributed to cannibalism, overall 90 *Paratrioza sinica* containing equal numbers (30 capita) of first–second instar nymphs, third–fifth instar nymphs and adults were offered to a leg-regenerated ladybug in a plastic container. Normal ladybugs were designed as control. The number and stage of *Paratrioza sinica* consumed after 24 h were recorded, then the preference and switching coefficients were predicted. Each treatment was replicated ten times (5♀:5♂) simultaneously.

2.6 Fecundity and hatchability

Four mating treatments were established when *Coccinella septempunctata* adults were ten days old: (1) regenerated ♀ × regenerated ♂, (2) normal ♀ × regenerated ♂, (3) regenerated ♀ × normal ♂ and (4) normal ♀ × normal ♂. The ladybugs were paired in a plastic container containing *Paratrioza sinica* diets. The male was removed from the container after copulation, and the female was fed on *Paratrioza sinica* diets refreshed daily through her life. The egg fecundity and hatching rate in each treatment were tested. Each treatment was replicated ten times simultaneously.

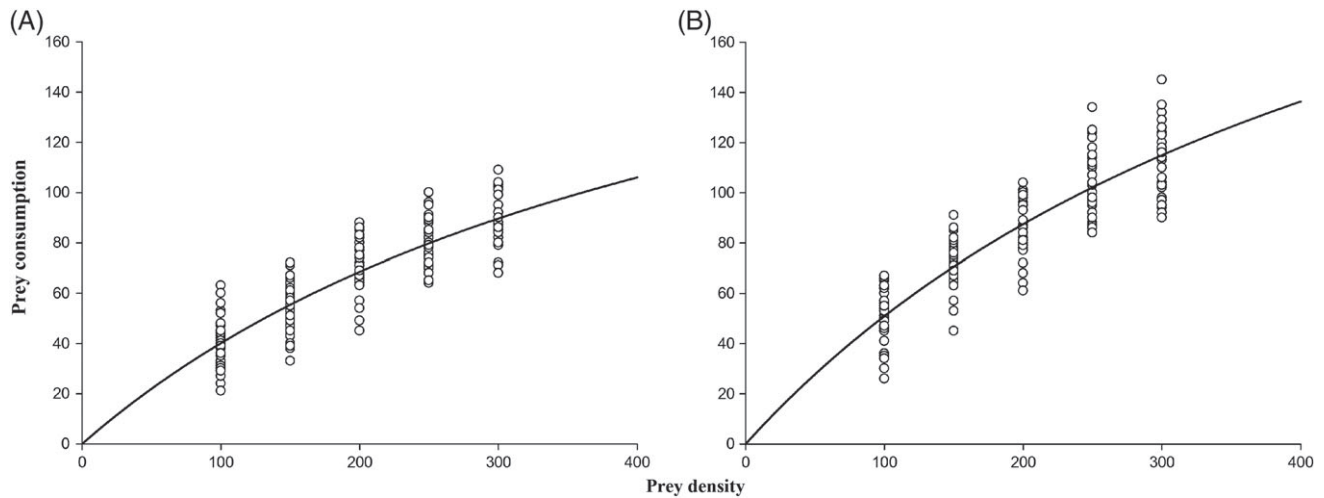


Figure 1. Prey consumption by *Coccinella septempunctata* over 24 h period. (A) Leg-regenerated ladybug, (B) normal ladybug. Each data point represents the observed *Paratrioza sinica* consumption. Functional response curves are fitted by the average consumption using the Holling's disc equation (Eqn 2).

2.7 Statistical analysis

2.7.1 Foraging ability

Functional responses are described using two-stage analysis.⁴⁴ Cubic logistic regression (Eqn 1) between proportion of prey consumed and prey initial density is firstly performed to determine the type of functional response⁴⁴:

$$\frac{N_a}{N_0} = \frac{\exp(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3)}{1 + \exp(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3)} \quad (1)$$

where N_a is the prey consumption, N_0 is the initial prey density, and P_0 , P_1 , P_2 , and P_3 are the intercept, linear, quadratic, and cubic coefficients, respectively. Negative or positive linear coefficients (P_1) from the regression indicate Type II or Type III curve.⁴⁴ Since the logistic regression analysis indicated that our data fit Type II in each case, further analyses were restricted to the Type II functional response. The Holling's disc⁴⁵ (Eqn 2) is used to model the relationship between the prey consumption (N_a) and initial prey density (N_0):

$$N_a = \frac{aTN_0}{1 + aT_h N_0} \quad (2)$$

where N_a and N_0 are described in Eqn 1, T is the total time which in this case is 24 h, a is the searching rate, and T_h is the handling time. A non-linear regression (NLR) procedure based on the Levenberg–Marquardt method (SPSS 20.0) is performed to estimate the parameters a and T_h . The starting values of a and T_h required by the NLR procedure are found by linearly regressing $1/N_a$ against $1/N_0$. The resultant y-intercept is the initial estimate of T_h and the reciprocal of the regression coefficient is an estimate of a .^{46,47}

2.7.2 Competitive ability

The experiment is to predict the coefficients of mutual interference between ladybugs in intraspecific competition. NLR analysis is performed to estimate parameters of an intraspecific competition model by fitting Eqn 3:⁴⁸

$$E = QP_d^{-m} \quad (3)$$

where E is the consumption rate, P_d is the predator density, m is the coefficient of mutual interference, and Q is the potential

maximum consumption rate. The values of Q and m are found by power-exponential regression E and P_d .

2.7.3 Prey preference

Preference coefficients of ladybugs are analyzed in the experiment. The preference model⁴⁹ (Eqn 4) is used to describe the preference of *Coccinella septempunctata* when *Paratrioza sinica* at various developmental stages coexist:

$$Q_i = (1 + C_i) / (1 - C_i) \times F_i \quad (4)$$

where Q_i is the proportion of prey i consumed by predators, F_i is the initial proportion of prey i , C_i is the preference coefficient of predators for prey i . Thus, $C_i = 0$ indicates no preference for prey i ; $0 < C_i < 1$ or $-1 < C_i < 0$ indicates positive or negative preference for prey i . Moreover, the switching behavior equation^{50,51} (Eqn 5) is displayed to evaluate switching levels in prey preference from normal to leg-regenerated ladybugs:

$$Q_i = \frac{1 + C'_i}{1 - C'_i} F_i^{1-S_i} \quad (5)$$

where Q_i and F_i are described in Eqn 4; C'_i is the preference coefficient of normal ladybugs on prey i ; S_i is the switching coefficient in prey preference from normal to leg-regenerated ladybugs. Thus, $S_i = 0$ indicates no switching behavior for prey i ; $0 < S_i < 1$ or $-1 < S_i < 0$ indicates positive or negative switching behavior for prey i .

Descriptive statistics were given as the mean values and standard errors of the mean. The preference or switching coefficients were compared to $C_i = 0$ or $S_i = 0$ using one-sample t -test. Fecundity and hatchability were analyzed using one-way analysis of variance (ANOVA) with the Tukey's HSD test of significance at the 5% level of statistical significance. Other data were analyzed using independent-sample t -tests. In all tests, P values < 0.05 were considered significant. All statistical analyses were conducted using the SPSS 20.0 software (IBM, Armonk, NY). Regression analyses were performed using SigmaPlot 12.0 software (Systat Software Inc., San Jose, CA, USA). All the correlation coefficients were obtained from SigmaPlot 12.0 software.

Table 1. Parameter estimate of Holling's disc equation for prey consumption of *Coccinella septempunctata*

Treatment	R^2	F	P	Equation	a	T_h (min)	Theoretical maximum prey consumption
Regenerated ladybug	0.993	429.126	<0.001	$N_a = 0.4844 N / (1 + 0.002070 N)$	0.48 ± 0.05	6.15 ± 1.25	258.2 ± 46.7
Normal ladybug	0.996	748.394	<0.001	$N_a = 0.6105 N / (1 + 0.001976 N)$	0.61 ± 0.04	4.66 ± 0.68	310.3 ± 38.4

Note: P is the probability that Holling's disc equation yields parameters; a is the searching rate; T_h is the handling time; theoretical maximum prey consumption is estimated when initial density tends to infinity.

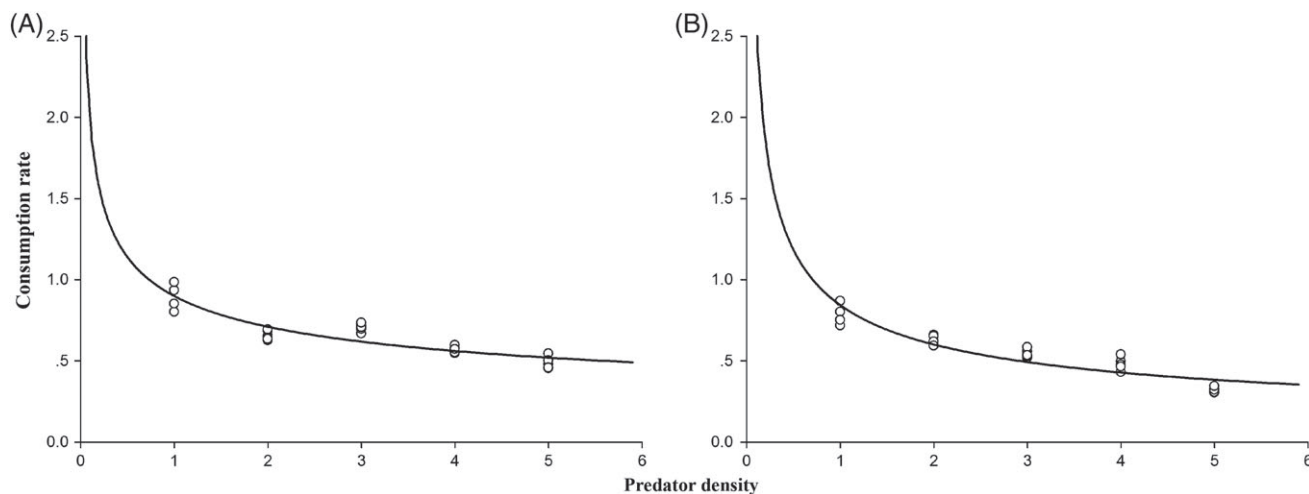


Figure 2. Consumption rates of *Coccinella septempunctata* in the intraspecific competition between (A) leg-regenerated and (B) normal individuals. Each data point represents the consumption rate of ladybugs. Curve is fitted by the average rates of *Paratrioza sinica* consumption via the intraspecific competition equation (Eqn 3).

3 RESULTS

3.1 Foraging ability

The number of consumed *Paratrioza sinica* increased significantly with the increased initial density (Fig. 1). The average consumption [mean \pm standard error (SE) = 66.9 ± 4.9] of leg-regenerated *Coccinella septempunctata* was significantly lower than that (85.1 ± 25.5) of control ($t_{28} = 2.231, P = 0.034$). According to parameter estimates from the cubic equation (Eqn 1) of proportion of prey consumption versus initial density, linear coefficients P_1 in leg-regenerated and normal ladybugs were -0.01929 and -0.02571 , respectively. Estimates of P_1 in both leg-regenerated ($P = 0.001$) and normal ($P = 0.014$) ladybugs were significantly negative, indicating that *Coccinella septempunctata* exhibited a Type II response to *Paratrioza sinica*. Data for all ladybugs fit the Holling's disc model well ($P < 0.05$), and the coefficients of determination (R^2) estimated by fitting Holling's disc equation were more than 0.95. The searching rate and handling time of leg-regenerated ladybugs predicted by Holling's disc equation non-significantly decreased ($t_4 = 1.655, P = 0.173$) and increased ($t_4 = 0.855, P = 0.441$), respectively. Moreover, slight reduction ($t_4 = 0.862, P = 0.437$) was examined in the theoretical maximum prey consumption of leg-regenerated ladybugs (Table 1).

3.2 Attachment ability

No significant difference occurred in body mass between leg-regenerated (31.1 ± 1.4 mg) and normal (32.3 ± 1.3 mg) ladybugs ($t_{38} = 0.62, P = 0.539$). However, the critical hanging weight (92.5 ± 4.2 mg) of leg-regenerated ladybugs was significantly lower than that (134.2 ± 6.9 mg) of the normal individuals

($t_{31.456} = 5.155, P < 0.001$). Based on the data earlier, a significantly lower attachment coefficient (3.0 ± 0.1) was detected in leg-regenerated ladybugs compared to that (4.2 ± 0.2) of control ($t_{28.465} = 6.143, P < 0.001$).

3.3 Competitive ability

Because of intraspecific competition, the consumption rate of ladybugs decreased as the introduced numbers of predator and prey were increased when the prey/predator ratio was kept at 60 for each pair of coccinellids (Fig. 2). The consumption rates at various predator-prey densities fit the intraspecific competition equation (Eqn 3) well ($P < 0.05$). Potential maximum consumption rates (Q) and coefficients of mutual interference (m) illustrated the intraspecific competition numerically. The potential maximum consumption rate of leg-regenerated ladybugs was lower than that of control ($t_8 = 1.38, P = 0.205$). Since the competitive inferiority attributed to the reduced consumption level, leg-regenerated ladybugs were more greatly hampered in competition with normal opponents ($t_8 = 4.643, P = 0.002$) (Table 2).

3.4 Prey preference

When *Paratrioza sinica* at various developmental stages co-existed, no preference was shown by leg-regenerated ladybugs for third-fifth instar nymphs ($t_4 = 0.838, P = 0.838$), whereas their significantly positive and negative preferences for first-second instar nymphs ($t_4 = 3.282, P = 0.03$) and adults ($t_4 = 3.426, P = 0.027$) of *Paratrioza sinica* were tested, respectively. Conversely, normal ladybugs exhibited markedly negative and positive preferences for the first-second instar nymphs ($t_4 = 6.356, P = 0.003$)

Table 2. Parameter estimate of intraspecific competition equation for consumption rate of *Coccinella septempunctata*

Treatment	R^2	F	P	Equation	Q	m
Regenerated ladybug	0.861	18.509	0.023	$E = 0.8426 P^{-0.4892}$	$84.3\% \pm 2.8\%$	$0.49 \pm 0.02^*$
Normal ladybug	0.873	20.583	0.02	$E = 0.8987 P^{-0.3406}$	$89.9\% \pm 3.0\%$	0.34 ± 0.02

Note: P is the probability that mutual interference equation yields parameters; Q is the potential maximum consumption rate; m is the coefficient of mutual interference. The asterisk indicates significant differences in parameters between leg-regenerated and normal ladybugs (independent t -tests, $P < 0.05$).

Table 3. Preference and switching levels of *Coccinella septempunctata* when *Paratrioza sinica* at various stages coexist

Prey i	C_i	C_i'	S_i
First–second nymphs	$0.15 \pm 0.05^*$	$-0.34 \pm 0.05^{**}$	$0.95 \pm 0.13^{**}$
Third–fifth nymphs	0.01 ± 0.04	-0.26 ± 0.10	0.53 ± 0.25
Adults	$-0.29 \pm 0.09^*$	$0.3 \pm 0.03^{**}$	$-1.14 \pm 0.20^{**}$

Note: C_i or C_i' is the preference coefficient of leg-regenerated or normal ladybugs on prey i ; S_i is switching coefficient in prey preference from normal to leg-regenerated ladybugs. Asterisks indicate coefficients significantly differ from $C_i = 0$ or $S_i = 0$ (one sample t -test, $*P < 0.05$; $**P < 0.01$).

and adults ($t_4 = 11.019$, $P < 0.001$), respectively, and no preference for the third–fifth instar nymphs ($t_4 = 2.614$, $P = 0.059$). Compared to normal coccinellids, the leg-regenerated individuals positively and negatively switched to first–second instar nymphs and adults of *Paratrioza sinica*, respectively (first–second instar nymphs, $t_4 = 7.289$, $P = 0.002$; third–fifth instar nymphs, $t_4 = 2.145$, $P = 0.099$; adults, $t_4 = 5.684$, $P = 0.005$) (Table 3).

3.5 Fecundity and hatchability

No significant difference was examined in the egg fecundity between leg-regenerated and normal females of *Coccinella septempunctata*, but the females paired with the leg-regenerated males obtained a notably higher fecundity compared to them paired with the normal males ($F_{3,196} = 45.975$, $P < 0.001$; Fig. 3(A)). Overall, the egg fecundity of leg-regenerated males increased by 15.3% compared to that of normal males. After oviposition, hatching rates of the three treatments were non-significantly lower than that of control ($F_{3,196} = 2.232$, $P = 0.086$; Fig. 3(B)).

4 DISCUSSION

4.1 Decreased prey consumption caused by cannibalism

Prey consumption by leg-regenerated *Coccinella septempunctata* was significantly reduced, and this impact was extended to other characteristics of predation such as searching rate, handling time and theoretical maximum consumption. This implies that, as newly regenerated legs are often less or even not functional,¹⁸ cannibalism not only damages the grasping ability of forelegs but also influences their sensory capability in spite of leg regeneration. Forelegs of predators are extremely important in determining direction and angle during prey location.^{52,53} Moreover, the impaired capture ability caused by cannibalism is likely to trigger a reduced prey acceptance of predators,⁵⁴ also reflecting the decreased consumption efficiency.

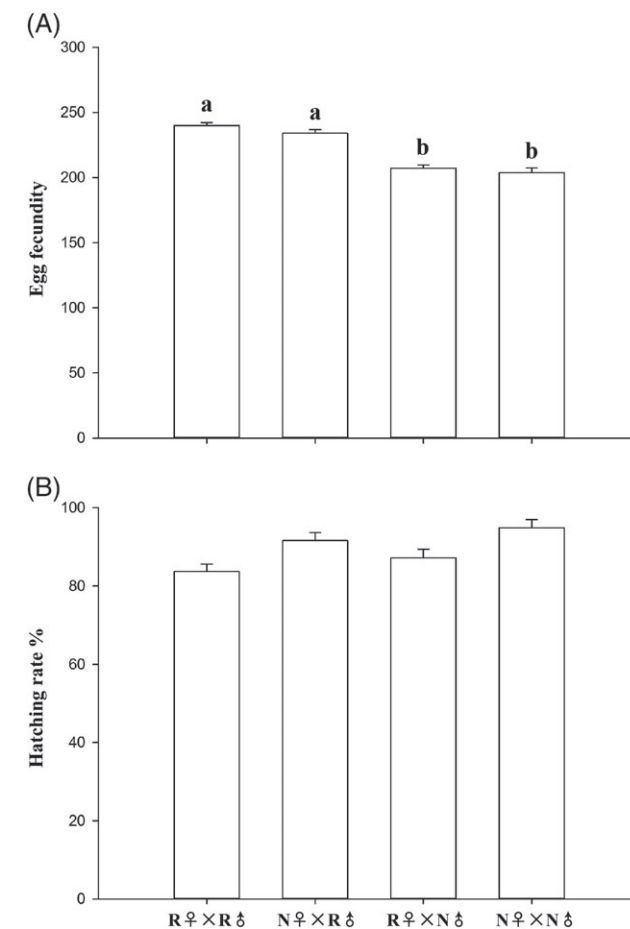


Figure 3. Reproductive performance of various mating pairs of regenerated (R) and normal (N) ladybugs (means \pm standard error). (A) Egg fecundity (number of eggs); (B) hatching rate (%). Different letters indicate significant differences among the treatments (mean separation by Tukey's HSD, $P < 0.05$).

4.2 Decreased attachment ability attributed to cannibalism

Feet are primary contact sites between animals and their environment via adhesive devices, helping their survival in complex habitats.⁵⁵ Since many attachment systems have developed independently in separated directions among animals with different sizes,^{24,25} small-sized animals such as insects tend to maximize friction on smooth surfaces.⁵⁶ The density of surface hairs positively correlates with the body weight of animals,²⁶ so the attachment coefficient is a more accurate reflection of the attachment ability. Our study shows that the attachment coefficient of leg-regenerated ladybugs was markedly lower than that of control, suggesting a decreased adhesive performance triggered by cannibalism. For the phenotype of the regenerated legs, they

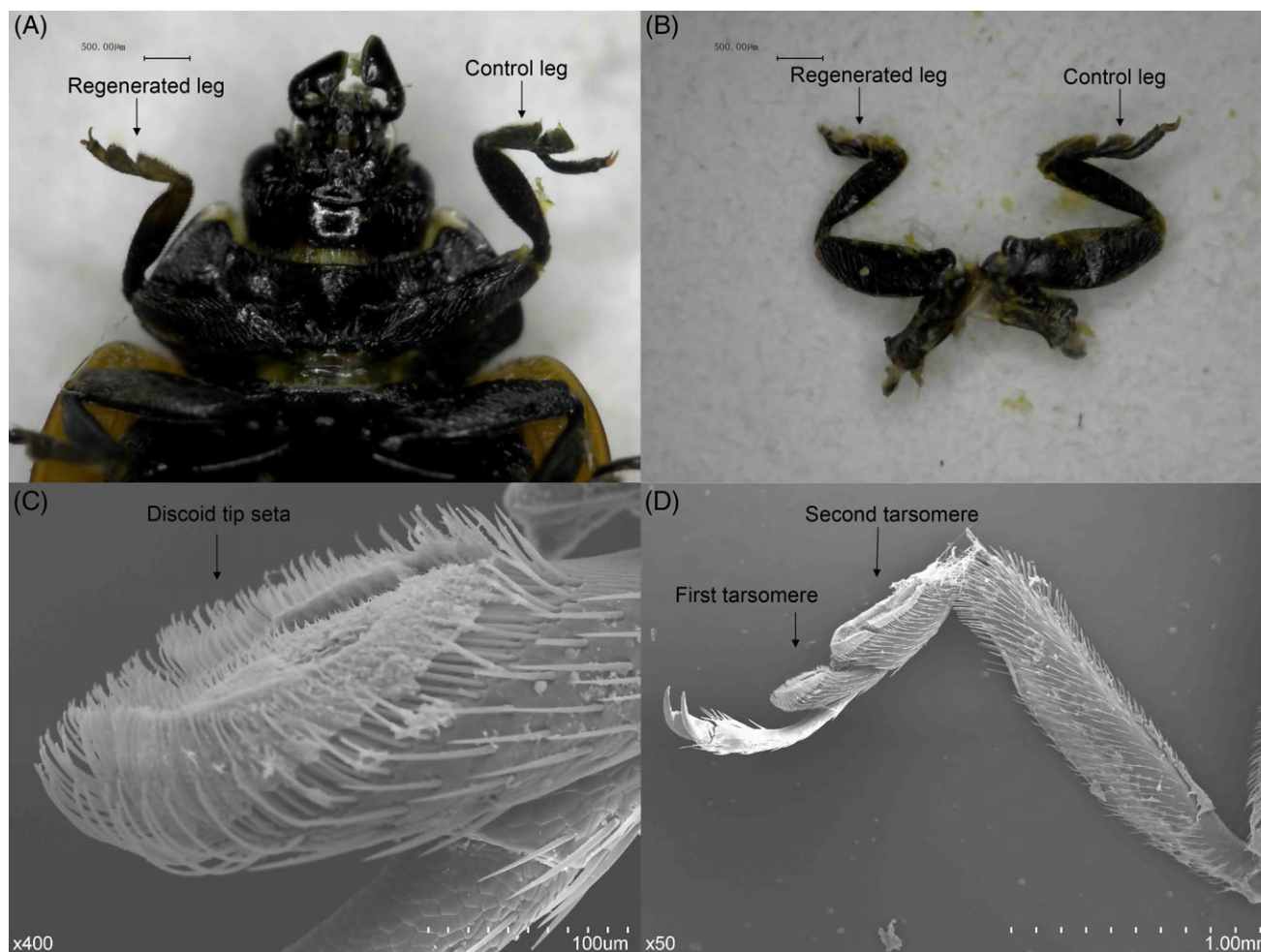


Figure 4. Phenotype of regenerated right foreleg and normal contralateral leg. (A) The regenerated leg was slightly shortened compared to the contralateral leg. (B) The curtailed segment was mainly at the distal end of the tarsus. Attachment system of regenerated leg via scanning electron microscope examination. (C) Discoid tip seta releasing adhesive secretions. (D) First and second tarsomeres served as adhesive pads.

(4.28 ± 0.13 mm) were slightly shortened by 4.5% compared to normal legs (4.53 ± 0.15 mm) (Fig. 4(A)), and curtailed segments were observed mainly at the distal end of the tarsus (decreased by 13.3%; Fig. 4(B)) as was reported previously.⁵ According to a scanning electron microscope examination, the discoid tip seta (Fig. 4(C)) served as adhesive pads at the ventral surfaces of the first and second tarsomeres (Fig. 4(D)) became less after leg regeneration, indicating the reduced attachment ability was triggered by the decreased tenent setae due to the curtailed tarsus under cannibalism situations. The spoon-shaped endplate is also served as a wet attachment system,²⁹ so discoid tip seta releasing adhesive secretions plays an important role in the attachment ability of ladybugs.

4.3 Decreased competitive ability leading to a preference for small-sized prey

Our study indicated that the foraging efficiency of ladybugs gradually decreased when predator–prey density was increased due to the increased intensity of intraspecific competition, which is consistent with the results of intraspecific competition in *Chilocorus bipustulatus*⁵⁷ and *Menochilus sexmaculatus*.⁵⁸ Our study also shows that leg-regenerated ladybugs were more inhibited because of their inferiority in competition with normal

ladybugs. Coccinellid is a digestive-limited predator, suggesting the maximum consumption rate is closely related to satiation.⁵⁹ Ladybugs focus on the predation when the consumption is below the satiation level, but once the consumption reaches the satiation level, they become inactive and thus are easily blocked by more active opponents.⁶⁰ Therefore, compared to the normal competitors, leg-regenerated ladybugs attributed to cannibalism are impeded more frequently because of the lower satiation level. The analogous result is also found in *Propylea quatuordecimpunctata*.⁶¹

Since impaired capabilities of newly regenerated legs,¹⁹ *Coccinella septempunctata* regenerating a foreleg was reluctant to capture big-sized adults of *Paratrioza sinica*, and significantly preferred the small-sized first–second instar nymphs. The switch from adults to young nymphs of *Paratrioza sinica* is due to the competitive inferiority of leg-regenerated ladybugs caused by cannibalism. In order to avoid massive energy losses and increase predation efficiency, the inferior competitors tend to choose prey easily captured and benefit by switching to small-sized prey after evaluating the cost of big-sized prey. Similar to coccinellids, many crustaceans and arachnids with leg regeneration are prone to switch to small-sized suboptimal prey.^{40,62}

4.4 Investment tradeoff between egg fecundity and functional recovery promoted by larval cannibalism

Although decreased foraging, attachment and competitive abilities were tested in leg-regenerated ladybugs, *Coccinella septempunctata* females mating with the leg-regenerated males produced more eggs. This implies that an investment tradeoff between offspring production and functional self-repair is likely to exist in leg-regenerated ladybugs caused by cannibalism, particularly the males. Paternal effects of leg-regenerated coccinellids potentially benefit egg fecundity.^{1,43} However, slightly lower proportions of emerging offspring were examined from treatment groups compared to that from the control group, suggesting deleterious genetics from damaged tissues or cells attributed to cannibalism possibly impaired egg hatchability and progeny survival,⁴² which deserves to be studied further.

As larval cannibalism occurs frequently in intensive rearing systems, it is common for ladybugs to lose legs,⁶³ and their various functional behaviors are partially impaired although the legs are regenerated in morphology. After larval cannibalism occurs, leg-regenerated ladybugs may be prone to make an investment tradeoff between egg fecundity and functional recovery, and this investment selection is meaningful in improving progeny production. Thus, larval cannibalism may potentially promote the offspring development of the biological control agent in complex environments.

4.5 Remaining questions and perspectives

In the study, only the right foreleg was damaged to imitate the result of larval cannibalism, the effects of cannibalism on the legs of various thoracic locations (foreleg, mid- or hind-leg) and sides (left or right leg) remained poorly understood. Regeneration of forelegs costs crickets *Gryllus bimaculatus* more time than that of hind-legs,¹¹ implying the distinguishable importance of different thoracic legs. When various thoracic legs are damaged simultaneously under cannibalism conditions, the priority of functional recovery among them deserves to be studied further. Even though forelegs are more functional in foraging and mating behaviors,^{64,65} different thoracic legs are damaged nearly equally in natural habitats.⁶⁶ Losing a mid- or hind-leg is also common in intensive rearing systems, so the effect of cannibalism on the mid- or hind-legs deserve to be considered as well. Besides, behavioral lateralization is identified in vertebrates,⁶⁷ and this asymmetry in insects draws increasing attention.⁶⁸ Mediterranean fruit fly *Ceratitis capitata* is found to preferentially utilize left legs or wings.⁶⁹ Thus, exploring behavioral lateralization is valuable to the further study on the potentially different effects of cannibalism on left-side and right-side legs of *Coccinella septempunctata*.

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REFERENCES

- 1 Michaud JP, A comparative study of larval cannibalism in three species of ladybird. *Ecol Entomol* **28**:92–101 (2003).
- 2 Schellhorn NA and Andow DA, Cannibalism and interspecific predation: role of oviposition behavior. *Ecol Appl* **9**:418–428 (1999).
- 3 Endo T, Bryant SV and Gardiner DM, A stepwise model system for limb regeneration. *Dev Biol* **270**:135–145 (2004).
- 4 Kumar A, Gates PB and Brockes JP, Positional identity of adult stem cells in salamander limb regeneration. *CR Biol* **330**:485–490 (2007).
- 5 Wu PX, Xiong XF, Li Z, Yan S, Liu XX and Zhang QW, Developmental continuity between larval and adult leg patternings in *Coccinella septempunctata* (Coleoptera: Coccinellidae). *Fla Entomol* **98**:193–199 (2015).
- 6 Brock RE and Smith LD, Recovery of claw size and function following autotomy in *Cancer productus* (Decapoda: Brachyura). *Biol Bull* **194**:53–62 (1998).
- 7 Amaya CC, Klawinski PD and Rormanowicz DR, The effects of leg autotomy on running speed and foraging ability in two species of wolf spider (Lycosidae). *Am Midl Nat* **145**:201–205 (2001).
- 8 Dodson GN and Beck MW, Precopulatory guarding of penultimate females by male crab spiders, *Misumenoides formosipes*. *Anim Behav* **46**:951–959 (1993).
- 9 Mariappan P, Balsundaram C and Schmitz B, Decapod crustacean chelipeds: an overview. *J Biosci* **25**:301–313 (2000).
- 10 Taylor PW and Jackson RR, Interacting effects of size and prior injury in jumping spider conflicts. *Anim Behav* **65**:787–794 (2003).
- 11 Bateman PW and Fleming PA, Sex and the single (eared) female: leg function, limb autotomy and mating history tradeoffs in field crickets (*Gryllus bimaculatus*). *Biol Lett* **2**:33–35 (2006).
- 12 Uetz GW, McClintock WJ, Miller D, Smith EI and Cook KK, Limb regeneration and subsequent asymmetry in a male secondary sexual character influences sexual selection in wolf spiders. *Behav Ecol Sociobiol* **38**:321–326 (1996).
- 13 Taylor PW, Roberts JA and Uetz GW, Compensation for injury? Modified multi-modal courtship of wolf spiders following leg autotomy of signaling appendages. *Ethol Ecol Evol* **18**:79–89 (2006).
- 14 Fleming PA, Muller D and Bateman PW, Leave it all behind: a taxonomic perspective of autotomy in invertebrates. *Biol Rev* **82**:481–510 (2007).
- 15 Stoks R, Autotomy shapes the trade-off between seeking cover and foraging in larval damselflies. *Behav Ecol Sociobiol* **47**:70–75 (1999).
- 16 Cooper WE, Shifted balance of risk and cost after autotomy affects use of cover, escape, activity, and foraging in the keeled earless lizard (*Holbrookia propinqua*). *Behav Ecol Sociobiol* **54**:179–187 (2003).
- 17 Danielson-Francois A, Fetterer CA and Smallwood PD, Body condition and mate choice in *Tegragnatha elongata* (Araneae, Tetragnathidae). *J Arachnol* **30**:20–30 (2002).
- 18 Vollrath F, Lyriiform organs on regenerated spider legs. *Bull Br Arachnol Soc* **10**:115–118 (1995).
- 19 Vollrath F, Leg regeneration in web spiders and its implications for orb weaver phylogeny. *Bull Br Arachnol Soc* **8**:177–184 (1990).
- 20 Arzt E, Gorb S and Spolenak R, From micro to nano contacts in biological attachment devices. *Proc Natl Acad Sci USA* **100**:10603–10606 (2003).
- 21 Gorb SN, Biological attachment devices: exploring nature's diversity for biomimetics. *Philos Trans R Soc A* **366**:1557–1574 (2008).
- 22 Moon MJ and Park JG, Fine structural analysis on the dry adhesion system of the jumping spider *Plexippus setipes* (Araneae: Salticidae). *Animal Cells Syst* **13**:161–167 (2009).
- 23 Autumn K, Dittmore A, Santos D, Spenko M and Cutkosky M, Frictional adhesion: a new angle on gecko attachment. *J Exp Biol* **209**:3569–3579 (2006).
- 24 Beutel RG and Gorb SN, Ultrastructure of attachment specializations of hexapods (Arthropoda): evolutionary patterns inferred from a revised ordinal phylogeny. *J Zoolog Syst Evol Res* **39**:177–207 (2001).
- 25 Irschick DJ, Austin CC, Petren K, Fisher RN, Losos JB and Ellers O, A comparative analysis of clinging ability among pad-bearing lizards. *Biol J Lin Soc* **59**:21–35 (1996).
- 26 Gao H and Yao H, Shape insensitive optimal adhesion of nanoscale fibrillar structures. *Proc Natl Acad Sci USA* **101**:7851–7856 (2004).

- 27 Federle W, Riehle M, Curtis ASG and Full RJ, An integrative study of insect adhesion: mechanics and wet adhesion of pretarsal pads in ants. *Integr Comp Biol* **42**:1100–1106 (2002).
- 28 Vötsch W, Nicholson G, Müller R, Stierhof YD, Gorb S and Schwarz U, Chemical composition of the attachment pad secretion of the locust *Locusta migratoria*. *Insect Biochem Mol Biol* **32**:1605–1613 (2002).
- 29 Gorb SN, The design of the fly adhesive pad: distal tenent setae are adapted to the delivery of an adhesive secretion. *Proc R Soc Lond B* **265**:747–752 (1998).
- 30 Niederegger S, Gorb S and Jiao Y, Contact behaviour of tenent setae in attachment pads of the blowfly *Calliphora vicina* (Diptera, Calliphoridae). *J Comp Physiol A* **187**:961–970 (2002).
- 31 Gorb SN and Gorb EV, Ontogenesis of the attachment ability in the bug *Coreus marginatus* (Heteroptera, Insecta). *J Exp Biol* **207**:2917–2924 (2004).
- 32 Autumn K and Peattie AM, Mechanisms of adhesion in geckos. *Integr Comp Biol* **42**:1081–1090 (2002).
- 33 Kim TW and Bhushan B, Adhesion analysis of multi-level hierarchical attachment system contacting with a rough surface. *J Adhesion Sci Technol* **21**:1–20 (2007).
- 34 Begon M, Harper JL and Townsend CR, Ecology : individuals, populations and communities. *Bioscience* **38**:424 (1996).
- 35 Hassell MP, *The Dynamics of Arthropod Predator-Prey Systems, in Monographs in Population Biology 13*. Princeton University Press, Princeton, NJ, pp. 174–183 (1978).
- 36 Dixon AFG, *Insect Predator-Prey Dynamics. Ladybird Beetles and Biological Control*. Cambridge University Press, Cambridge (2000).
- 37 Maginnis TL, Leg regeneration stunts wing growth and hinders flight performance in a stick insect (*Sipylodea sipylus*). *Proc Biol Sci* **273**:1811–1814 (2006).
- 38 Smith LD, The impact of limb autotomy on mate competition in Blue Crabs *Callinectes sapidus* Rathbun. *Oecologia* **89**:494–501 (1992).
- 39 Rypstra AL, Schmidt JM, Reif BD, DeVito J and Persons MH, Tradeoffs involved in site selection and foraging in a wolf spider: effects of substrate structure and predation risk. *Oikos* **116**:853–863 (2007).
- 40 Brueseke MA, Rypstra AL, Walker SE and Persons MH, Leg autotomy in the wolf spider *Pardosa milvina*: a common phenomenon with few apparent costs. *Am Midl Nat* **146**:153–160 (2001).
- 41 Saxena S, Mishra G and Omkar O, Does regeneration ability influence reproductive fitness in *Menochilus sexmaculatus*, (Coleoptera: Coccinellidae)? *J Asia Pac Entomol* **19**:829–834 (2016).
- 42 Wang S, Tan XL, Michaud JP, Shi ZK and Zhang F, Sexual selection drives the evolution of limb regeneration in *Harmonia axyridis* (Coleoptera: Coccinellidae). *Bull Entomol Res* **105**:245–252 (2015).
- 43 Mirhosseini MA, Michaud JP, Jalali MA and Ziaaddini M, Paternal effects correlate with female reproductive stimulation in a polyandrous ladybird. *Cheilomenes sexmaculata B Entomol Res* **104**:480–485 (2014).
- 44 Juliano SA, Nonlinear curve-fitting: predation and functional response curves, in *Design and Analysis of Ecological Experiments*, 2nd edn, ed. by Scheiner SM and Gurevitch J. Oxford University Press, New York, NY, pp. 178–196 (2001).
- 45 Holling CS, Some characteristics of simple type of predation and parasitism. *Can Entomol* **91**:385–398 (1959).
- 46 Livdahl TP and Stiven AE, Statistical difficulties in the analysis of predator functional response data. *Can Entomol* **115**:1365–1370 (1983).
- 47 Watson DM, Du TY, Li M, Xiong JJ, Liu DG, Huang MD et al., Functional responses of, and mutual interference in *Aleurodothrips fasciapennis* (Franklin) (Thysanoptera: Phlaeothripidae) and implications for its use as a biocontrol agent. *Gen Appl Entomol* **29**:31–37 (2000).
- 48 Hassell MP and Varley GC, New induction population model for insect parasites and its bearing on biological control. *Nature* **223**:1113–1137 (1969).
- 49 Zhou JZ and Chen CM, Predation of wolf spider *Lycosa pseudoannulata* to brown planthopper *Nilaparvata lugens* and simulation models. III. Selective predation. *Acta Ecol Sin* **7**:228–237 (1987).
- 50 Zhou JZ and Chen CM, Studies on predation and simulation models of wolf spider *Lycosa pseudoannulata* to brown planthopper *Nilaparvata lugens*. II. Simulation model and stability analysis of the monopredator-monoprey species system. *Acta Ecol Sin* **6**:238–247 (1986).
- 51 Zhou JZ and Chen CM, Studies on predation and simulation models of wolf spider *Lycosa pseudoannulata* to brown planthopper *Nilaparvata lugens*. IV. Simulation model and stability analysis about the model of monopredator-two prey species system. *Acta Ecol Sin* **7**:349–358 (1987).
- 52 Hergenroder R and Barth FG, Vibratory signals and spider behavior: how do the sensory inputs from the eight legs interact in orientation? *J Comp Physiol* **152**:361–371 (1983).
- 53 Klarner D and Barth F, Vibratory signals and prey capture in web spiders (*Zygiella x-notata*, *Nephila clavipes*). *J Comp Physiol* **148**:445–455 (1982).
- 54 Nentwig W and Wissel C, Comparison of prey lengths among spiders. *Oecologia* **68**:595–600 (1986).
- 55 Federle W, Why are so many adhesive pads hairy? *J Exp Biol* **209**:2611–2621 (2006).
- 56 Kesel AB, Martin A and Seidl T, Adhesion measurements on the attachment devices of the jumping spider *Evarcha arcuata*. *J Exp Biol* **206**:2733–2738 (2003).
- 57 Hattingsh V and Samways MJ, Absence of intraspecific interference during feeding by the predatory ladybirds *Chilocorus* spp. (Coleoptera: Coccinellidae). *Ecol Entomol* **15**:385–390 (1990).
- 58 Agarwala BK, Bardhanroy P, Yasuda H and Takizawa T, Effects of conspecific and heterospecific competitors on feeding and oviposition of a predatory ladybird: a laboratory study. *Entomol Exp Appl* **106**:219–226 (2003).
- 59 Papanikolaou NE, Milonas PG, Demiris N, Papachristos DP and Matsinos YG, Digestion limits the functional response of an aphidophagous coccinellid. *Ann Entomol Soc Am* **107**:468–474 (2014).
- 60 van Gils JA and Piersma T, Digestively constrained predators evade the cost of interference competition. *J Anim Ecol* **73**:386–398 (2004).
- 61 Papanikolaou NE, Demiris N, Milonas PG, Preston S and Kyraios T, Does mutual interference affect the feeding rate of aphidophagous coccinellids? A modeling perspective. *PLoS ONE* **11**:e0146168 (2016).
- 62 Juanes F and Smith LD, The ecological consequences of limb damage and loss in decapod crustaceans: a review and prospectus. *J Exp Mar Biol Ecol* **193**:197–223 (1995).
- 63 Osawa N, Sibling cannibalism in the ladybird beetle *Harmonia axyridis*: fitness consequences for mother and offspring. *Res Popul Ecol* **34**:45–55 (1992).
- 64 Rovner J, Morphological and ethological adaptations for prey capture in wolf spiders (Araneae, Lycosidae). *J Arachnol* **8**:201–215 (1980).
- 65 Scheffer SJ, Uetz GW and Stratton GE, Sexual selection, male morphology, and the efficacy of courtship signaling in two wolf spiders (Araneae: Lycosidae). *Behav Ecol Sociobiol* **38**:17–23 (1996).
- 66 Wrinn KM and Uetz GW, Impacts of leg loss and regeneration on body condition, growth and development time in the wolf spider *Schizocosa ocreata*. *Can J Zool* **85**:823–831 (2007).
- 67 Rogers JL and Andrew RJ, *Comparative Vertebrate Lateralization*. Cambridge University Press, Cambridge, UK, pp. 94–125 (2002).
- 68 Frasnelli E, Vallortigara G and Rogers L, Left-right asymmetries of behaviour and nervous system in invertebrates. *Neurosci Biobehav R* **36**:1273–1291 (2012).
- 69 Benelli G, Donati E, Romano D, Stefanini C, Messing RH and Canale A, Lateralisation of aggressive displays in a tephritid fly. *Sci Nat* **102**:1251 (2015).