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# Diets structure of a common lizard *Eremias argus* and their effects on grasshoppers: Implications for a potential biological agent

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## ABSTRACT

Outbreaks of grasshoppers (Orthoptera: Acrididae) often cause serious ecological damage. Recently, there has been interest in using natural enemies of grasshopper for their biological control. This study examined the biology and predation on grasshoppers by *Eremias argus*, a common lizard in Inner Mongolia. Its developmental duration and life history are consistent with adaptation to grasshopper phenology. The diet structure of *E. argus* included grasshoppers, beetles, ants, leafhoppers, moths, bees and spiders. A positive correlation between *E. argus* population numbers and grasshopper population numbers was highly significant. We built models describing the functional response and intraspecific competition for *E. argus* adults. The functional feeding response of *E. argus* on grasshopper was classed as Holling type III. The maximum number of grasshoppers consumed by female and male adults were 11.2 and 7.0 individuals per day, respectively. Predatory ability was strongest in female adults, with the second and third instars of *Oedaleus asiaticus* preferred. The predation ratios*(E)* of *E. argus* on grasshoppers declined with increasing lizard density, especially for female adult *E. argus*. *E. argus* could play an important role in grasshopper control and maintaining a population of *E. argus* in the grassland ecosystem could effectively control low-density grasshopper populations on grassland. Suggestions on how *E. argus* could fit into an IPM programme for biological control of grasshopper are discussed.

## 1. Introduction

Grasshoppers, including locusts, have long plagued humans, especially in grasslands and adjacent agricultural areas (Lecoq and Sukirno, 1999; Hunter, 2005). Locusts typically undergo dramatic population cycles, whereby they exist for many years at low or medium population densities, followed by an "outbreak" phase, where they greatly increase their numbers. During outbreaks, locusts and grasshoppers can devastate crops and grasslands, causing massive economic, social, and ecological disruptions, contributing to famine, disease and war (Carruthers et al., 1993; Lomer et al., 2001).

Numerous techniques have been used to control locusts and grasshoppers, with insecticide treatment preeminent (Lomer et al., 2001). However, insecticide use has several problems, including pollution of food and environment and non-target impacts on natural enemies (Peveling, 2001; Yu and Ji, 2007). Therefore, less harmful and more efficient measures for grasshopper control are urgently needed (Peveling, 2001). This sentiment is reflected in increasing interest in the use of natural enemies to control grasshoppers (Greathead, 1991; Carruthers et al., 1993; Lomer and Prior, 2001). Biological control can have many advantages, such as minimal ecosystem disruption, no toxic residues that can create environmental pollution and often can be applied at a low cost (Flexner et al., 1986; Peveling et al., 1999). Also some biological control agents can persist in the host's environment over multiple years, providing a permanent no-cost control option (Lomer et al., 2001). In China, research has examined grasshopper predation by birds, frogs, ground beetles and a parasitoid wasp *Telenomus* sp. (Hymenoptera: Scelionidae) (Ji et al., 2008). For example, protection of the migratory rosy starling *Sturnus roseus* L. (Passeriformes: Sturnidae) is a popular conservation measure for grasshopper control in China (Yu and Ji, 2007; Ji et al., 2008; Wang et al., 2010). However, further research on the natural enemies of grasshoppers could provide new ideas for their biological control.

In this paper, we examine the potential of the lizard *Eremias argus* (Peters) (Squamata: Lacertidae) as a biological control agent against pest grasshoppers in Inner Mongolia, China. This species is widely distributed in southern Inner Mongolia and in the provinces of Shanxi,

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Hebei, Shandong, Anhui and northern Jiangsu in China, and in some areas is the most common lizard species (Chen, 1991; Jin et al., 2012). Its basic biology has been documented, including species range, niche differentiation, reproduction, and thermal biology (Shine, 2003; Luo et al., 2005; Guo and Xu, 2008). However, its biological control potential for grasshoppers has not been studied.

The present study examined and investigated the phenology, population density, diet, feeding rates, and functional response of *E. argus*, with the aim of providing a theoretical basis for the protection of *E. argus* and its use as a biological control organism for grasshoppers.

## 2. Materials and methods

### 2.1. Study sites and animal collection

The study was conducted at (E 109.314°–E 110.145°, N 42.132°–N 42.240°) in the grassland of Damao County of Inner Mongolia (Fig. 1), a semi-desert grassland ecosystem with typically less than 20% vegetation coverage. Rapid grassland degradation has led to reduced biodiversity, decreased productivity and, in some cases, desertification, likely due to anthropogenic factors, specially overgrazing by livestock (Han et al., 2008). The main vegetation consists of two grasses, *Stipa krylovii* Roshev and *Caragana microphylla* Lam., which comprise ~82% of plant individuals (Chen and Wang, 2000). The rainfall from spring through to fall is generally below 220 mm and the annual average annual temperature is 2.1 °C. Although this ecosystem maintains a rich grasshopper fauna (59 species), three species dominate, *Oedaleus asiaticus* B. Bienko, *Bryodema luctuosum* Stoll, and *Myrmeleotettix palpalis* (Zubowsky), comprising ~88% of all grasshopper individuals in this area (Cease et al., 2012).

We collected nymphal grasshoppers of the three species by sweep net, in June 2011, then temporarily stored them outdoors in aluminum-frame cages (size: 1 m × 1 m × 1 m), covered with fine mesh netting. Caged grasshoppers were fed ~50 g of fresh-cut *S. krylovii* Roshev and ~50 g of fresh-cut *Leymus chinensis* (Trin.) Tzvel., daily. Female and male adult *E. argus* lizards were collected in June, 2011. Lizards were found by visual searching, and then captured by placing a sweep net over them. Thirty captured lizards were temporarily maintained outdoors in similar 1-m$^3$ metal-frame cages covered with netting (6 individuals/cage). Soil and rocks were placed in the bottom of the cage to provide a substrate and nesting site. All captive lizards were given live insect food daily, including grasshopper nymphs, beetles, and ants. Collections were authorized by the Grassland Workstation of Damao County and the Institute of Plant Protection, Chinese Academy of Agricultural Sciences.

**Fig. 1.** Map indicating location of field site is in the middle of Inner Mongolia (northern China).

### 2.2. E. argus biology and relationship with grasshoppers

To document *E. argus* phenology and daily behavior, we recorded the presence or absence of lizards, time of day, and their location (ground, vegetation, in sun, or in shade) during our field activities between May and October, 2010–2014. We also set up three 1-m$^3$ outdoor rearing cages, each with a 30-cm layer of field-collected soil and some rocks on the bottom. Each cage contained a pair of adult *E. argus*, provided with an ample insect diet of collected grasshoppers, beetles and ants, which was added to the cages at 0800 h each day. These captive lizards and their offspring were observed periodically for 3 years, with daily activity, feeding behavior, mating, oviposition, hatching, and survival recorded.

We used the Jolly–Seber stochastic method (Southwood, 1978) to estimate *E. argus* population density, in June 2013. We captured *E. argus* in each of our four, 4-km$^2$ plots (Nandian, Radar Station, Yangchangdong, and Huanghuatan). Each plot was subdivided into other four square areas of 1-km$^2$, in which we collected three-five *E. argus* in each square areas of 1-km$^2$. A total 16 of 1-km$^2$ were surveyed (four square areas of 1-km$^2$ per plot). Captured *E. argus* were marked on the back of the neck with a red marker pen, then rereleased at the point at which they had been collected. After three days, each of the 16 sites were resurveyed and 3–10 individuals collected with the number of marked *E. argus* recorded and rereleased.

We estimated the density of all grasshoppers in each of our 16 square areas of 1-km$^2$ of four plots on 13 June 2013. For each square areas of 1-km$^2$, we started at the center of the plot, then walked 500 m in each of the four cardinal directions, and conducted a standard sweep-net survey whereby 100 sweeps were made, with the net inspected after every 100 sweeps and the number of grasshoppers recorded. The number of grasshoppers collected was averaged for the four samples in each plot to derive an average grasshopper density for each plot.

To study *E. argus* food preference, we carefully observed food selection in our caged lizards. We also captured nine female adult *E. argus* from Radar Station and nine from Rinandian in mid-July, 2013, euthanized them, dissected their stomachs, and recorded their arthropod contents, as per Ji et al. (2008).

### 2.3. Functional response of E. argus

We examined the functional response of adult male and female *E. argus* to increasing availability of grasshopper prey. Lizards were collected from the wild in mid-June 2013, and immediately tested. Each lizard was maintained individually, outdoors, in a 1 m × 1 m × 1 m cage, containing fresh grasshopper-host plants. Lizards were starved for 24 h then subjected to one of three experimental designs: (1) Females were offered 5, 10, 15, 20, 25, 30, or 35 2nd–3rd instar *O. asiaticus* grasshopper nymphs; (2) Males offered 2, 4, 6, 8, 10, 12, or 14 2nd–3rd instar *O. asiaticus* grasshopper nymphs; and (3) Female *E. argus* offered 5, 7, 9, 11, 13, 15, or 17 4th instar grasshoppers per cage. In each case, we recorded the number of *O. asiaticus* consumed within 24 h. Each treatment was repeated four times. Upon completion of this experiment, all lizards were released back into the wild.

### 2.4. Intraspecific interference of E. argus

The density of predators can affect the predation ratios. With increasing predator density, intraspecific competition of predators is more likely to increase when preying, resulting in a subsequent decrease in predation ratios (Hassell and Varley, 1969; Ding, 1994). To explore the intraspecific competition between female and male adults of

*E. argus* on feeding grasshopper, respectively, we fed different densities of either female or male *E. argus* (1, 3, 5, 7, 9, and 11 individuals per cage) with fifty 2nd–3rd instar *O. asiaticus* in aluminum-frame cages (as described above). The lizards and *O. asiaticus* were placed in each cage simultaneously along with 30 g fresh *S. krylovii* as food for the grasshoppers. The lizards were starved for 24 h prior to setting up the experiment, and the number of *O. asiaticus* consumed were recorded after 24 h. The experiment was carried out in mid-June 2013, with each treatment replicated four times. After finishing this experiment, all lizards were released from the area they had been collected from.

## 3. Data analysis

We used Student's *t*-test to compare grasshopper numbers between populations of *E. argus*. *E. argus* population numbers were calculated by the formula (Southwood, 1978; Ge, 2007): $N = \frac{Mn}{m}$, where *M* is the initial numbers of marked *E. argus* (eight individuals in each squared area of 1 $\text{km}^2$), *m* is the recaptured numbers of marked *E. argus*, and *n* was the total numbers of *E. argus* at recapture times (20 individuals in each area). The equation of Holling type III, $N_a = \frac{bN_tT}{1+cN_t+bN_t{}^2T_h}$ was used to determine the functional responses of predators, where *b* and *c* were constant, *T* was experimental time of functional response (here was 24 h), $N_t$ is the initial numbers of prey, $N_a$ is the numbers of prey consumed, and $T_h$ was handling time. The equation $E = QP_t{}^{-m}$, $[E = N_a/(P_tN_t)]$ was used to calculate the intraspecific interference, where *m* is the mutual interference parameter, $P_t$ is the density of predators, *E* is the predation rate, *Q* is the seeking parameter, $N_t$: the initial numbers of prey (here was 50 individuals), and $N_a$: the numbers of prey consumed (Hassell and Varley, 1969; Ding, 1994). In order to investigate if populations of lizards and grasshoppers were related, we used linear-regression analysis to compare grasshopper density vs. lizard density in our four plots. Data was analyzed using the statistical software package IBM SPSS Statistics (SPSS 17.0).

## 4. Results

### 4.1. E. argus biology and relationship with grasshoppers

Observation of both caged and field populations over the three years showed that *E. argus* hibernated 20–25 cm underground during the winter months. Hibernation ended in late May when midday basking was observed. *E. argus* remained active through September, with peak activity observed in the months of June and July. Mature female adults began to lay eggs in mid-July. The eggs were oval with the initial yellow yoke later changing to white. Females laid batches of two to four eggs in the nest, which began to hatch after fifteen days. The juveniles live with the adults in the same nest for approximately 12 months, leaving when the adults began to lay a new clutch of eggs. It takes three years to reach sexual maturity. In mid-June, adult *E. argus* were observed to consume a substantial proportion of grasshopper species that emerged in the grassland, including *O. asiaticus, Dasyhippus barbipes* (Fischer-Waldheim) and *Myrmeleotettix palpalis* (Zubowsky).

Linear-regression analysis of lizard density and grasshopper density in four areas, showed that *E. argus* numbers were strongly correlated with grasshopper density ($F = 538.255$, $P < 0.0001$, $R^2 = 0.9746$) (Fig. 2).

The stomach contents of *E. argus* included seven kinds of arthropod taxa, namely grasshoppers, beetles, ants, leafhoppers, moths, bees and spiders (Fig. 3). *E. argus* mainly preyed on grasshoppers (23.4% in Radar Station, 39.73% in Rinandian), beetles (36.17%, 36.99%) and ants (29.79%, 8.22%). Meanwhile, we found that the number of grasshopper consumed by *E. argus* at Rinandian where grasshopper density was 38 individuals per 100 sweep net was significantly greater than Radar Station where grasshopper density was 19 individuals per 100 sweep net (Fig. 4, *t*-test; $F = 15.1$, $P = 0.0082$). This was presumably related to the markedly greater density of grasshoppers at Rinandian.

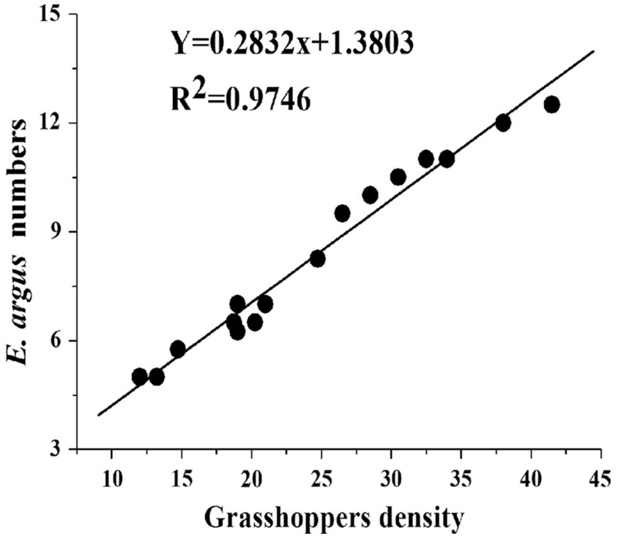

**Fig. 2.** Realtionship of *E. argus* population numbers (numbers of individual per 1 $\text{km}^2$) and grasshopper density (numbers of individual per 100 sweeps) in Damao County.

### 4.2. Functional response of E. argus to O. asiaticus

From the test of functional response, as vertebrate, functional responses of female and male adults of *E. argus* all corresponded to Holling type III (Holling, 1959) — a "S″ curve model (Fig. 5, Female for

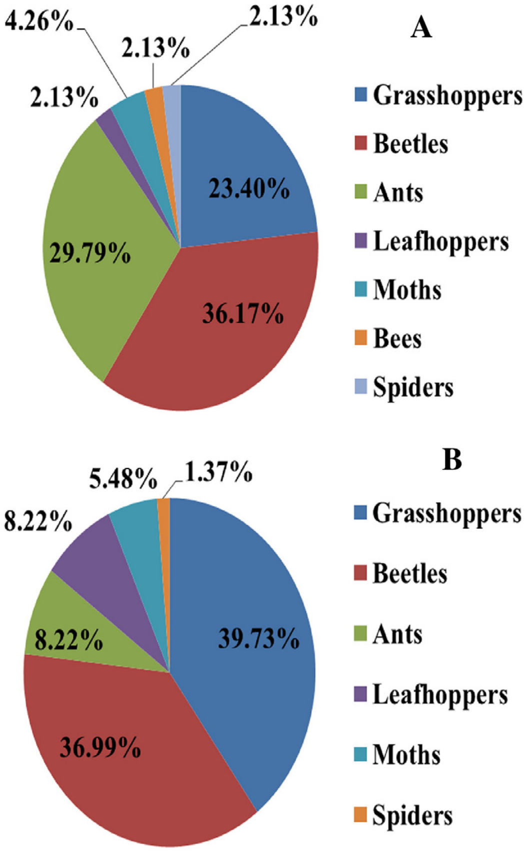

**Fig. 3.** Diet structure of *E. argus* in Radar Station (A) and Rinandian (B) indicating the different taxa consumed at these locations.

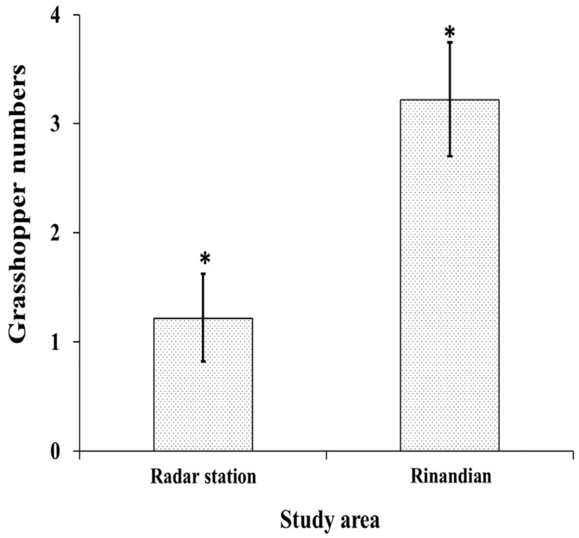

**Fig. 4.** Mean (± SE) numbers of grasshoppers recorded in the stomach of *E. argus* at Radar Station and Riniandian. '*' indicates that the value of bar chart are significantly different at P < 0.05.

the 2nd–3rd instars of *O. asiaticus*: $N_a = \frac{0.8929N_t}{1+0.0268N_t+0.0007{N_t}^2}$, $F = 579.368$, $P < 0.0001$, $R^2 = 0.9789$; Male for the 2nd–3rd instars: $N_a = \frac{0.5859N_t}{1\text{-}0.0725N_t+0.0061{N_t}^2}$, $F = 81.949$, $P < 0.0001$, $R^2 = 0.8677$; Female for the 4th instar: $N_a = \frac{0.7456N_t}{1\text{-}0.0623N_t+0.0042{N_t}^2}$, $F = 215.353$, $P < 0.0001$, $R^2 = 0.9643$). All functional response curves (Fig. 5) that reflected the relationship of prey consumed in relation to initial prey density were observed to go through three phases, namely an accelerating, deceleration and asymptotic phases. When the asymptotic phase was reached, the numbers of prey consumed did not change with further increases of prey density. The maximum number of prey consumed at saturation was found to be highest in female adults with feeding on the 2nd–3rd instars of *O. asiaticus* (11.2 individuals per day), followed by male adults (7.0 individuals per day). The highest number of feeding on the 4th instar of female adults was 11.1 individuals per day. Clearly, predatory ability was strongest in female adults, with the 2nd–3rd instars of *O. asiaticus* preferred.

### *4.3. Intraspecific interference of E. argus*

This study showed that *E. argus* exhibited intraspecific interference (Fig. 6) consistent with competition theory (Hassell and Varley, 1969; Ding, 1994). For female *E. argus* the predation rate ($E$) was: $E = 0.2722\ {P_t}^{-0.9122}$, $F = 45.799$, $P = 0.002$, $R^2 = 0.9197$ and for males $E = 0.1523\ {P_t}^{-0.6867}$, $F = 167.840$, $P < 0.0001$, $R^2 = 0.8547$). In other words, the predation rate of *O. asiaticus* decreased with increasing numbers of female and male adults, respectively. From the parameter estimation, the intensity of *E. argus* intraspecific competition was greater for female than males, with the mutual interference parameter ($m$) of female adults ($m = 0.9122$) was higher than male adults ($m = 0.6867$).

## 5. Discussion

Over the past 20 years, grasslands have been increasingly affected by drought, overgrazing, degradation, desertification and salinization (Han et al., 2008). Consequently, wildlife populations, including the natural enemies of grasshoppers, have significantly declined, resulting in grasshopper plagues (Cease et al., 2012). Therefore, research on beneficial wildlife and natural grasshopper predators is required to facilitate their protection and potential application in biocontrol. In China, many natural grasshopper enemies have been explored with respect to their biology and predation (Ji et al., 2008), however there is a paucity of research that has evaluated the ability of these natural enemies to regulate grasshopper populations. We studied the developmental duration, biology of *E. argus* to provide a theoretical basis for its protection and application. Understanding the functional response and intraspecific interference of *E. argus* in relation to grasshopper populations provides key data to determine the potential of this lizard species to be a useful natural enemy in an integrated pest management programme.

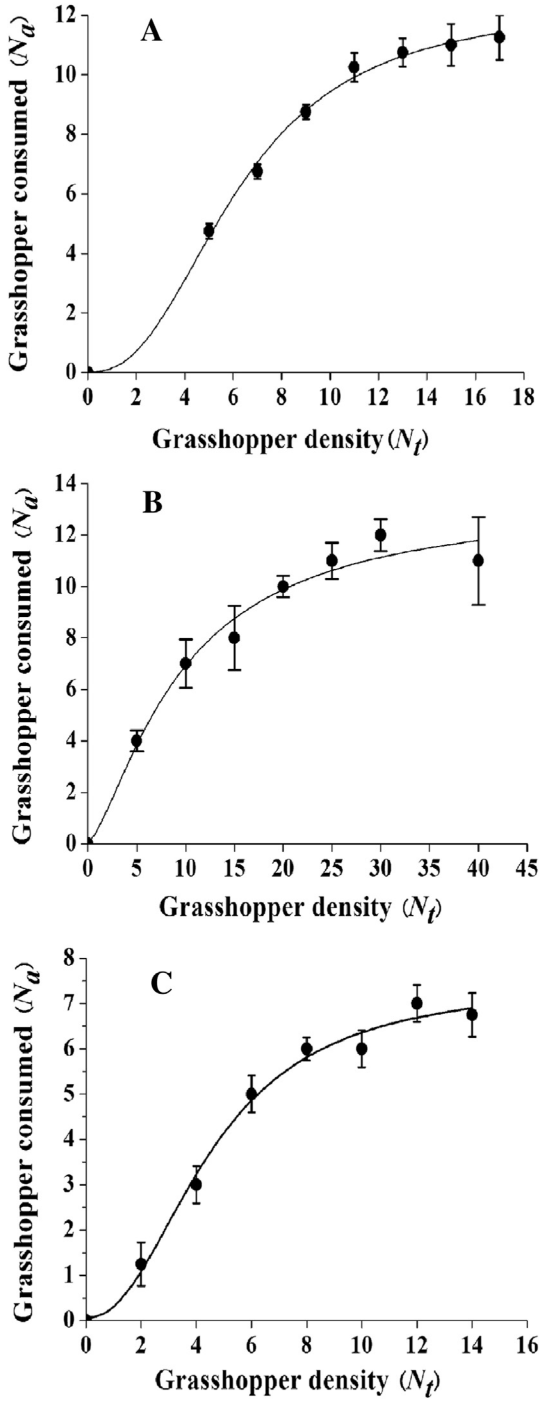

**Fig. 5.** Functional response curves for predation of *E. argus* on *O. asiaticus*. The "S" curves described the relationship between prey density (grasshopper density) ($N_t$) and the number of prey (grasshopper) consumed ($N_a$) (mean ± SE) by *E. argus* for (A) Predation of female adults on 4th instar of *O. asiaticus*; (B) Predation of female adults on 2nd-3rd instar of *O. asiaticus* and (C) Predation of male adults on 2nd-3rd instar of *O. asiaticus*.

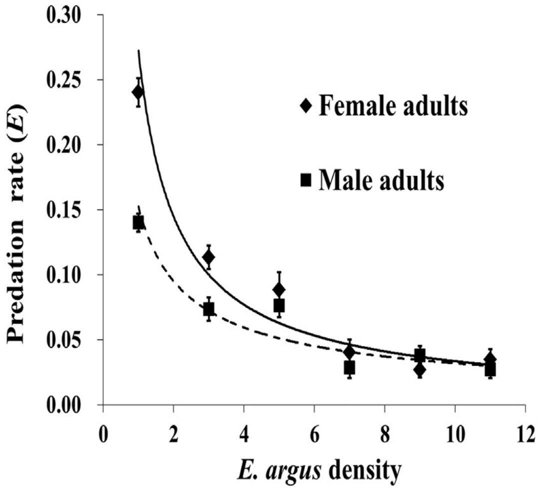

**Fig. 6.** Intraspecific interference of *E. argus*. Changes of predation rate (*E*) with increasing predator (*E. argus*) density (mean ± SE) (numbers of individuals per cage) for female and male adults.

This study clearly showed that *E. argus* preyed on large numbers of grasshoppers within 24 h, with feeding rate closely associated with grasshopper density. The functional feeding response of *E. argus* on grasshoppers was categorized as Holling type III, which is consistent with the functional response curves generally observed for vertebrate species (Holling, 1959). Adult female lizards require more energy and nutrients than males to meet their reproductive needs, and was reflected in the higher feeding rate of females.

There was also an intraspecific interference in *E. argus*. Wen (2007) found that the intraspecific competition between juveniles and adults was very low because they feed upon prey of different sizes but interspecific competition with other lizard species such as *Phrynocephalus frontalis* was strong. However, intraspecific competition among male adults and female adults requires a further investigation. In our research, intraspecific competition between the sexes increased with an increase in the respective number. Compared with male adults, the predation rate (*E*) of female adults declined considerably while intraspecific competition was greater presumably because they required more energy for reproduction. Although intraspecific competition for *E. argus* when preying grasshopper was existence, but in the wild, the living space of *E. argus* is an open rather than a small confined space. The intraspecific competition would not be so strong. Meanwhile, the natural selection based on foods and enemies also could control the population quantity of *E. argus* within limits. So intraspecific interference could not effect the use of *E. argus* as a biocontrol organism.

The peak in the activity of *E. argus* coincided with the period of emergence of 2nd–3rd instar *O. asiaticus*, at which size this grasshopper becomes a suitable prey (Feng and Zhou, 2010). The polyphagous diet of *E. argus* would allow it to sustain its population on alternative hosts to some extent (Wen, 2007), and when the population of other grasshopper species increases, *E. argus* could potentially be useful for the control of these grasshopper populations by transferring to these species found in the same environment. Thus, maintaining populations of *E. argus* at a certain level could effectively control low-density grasshopper populations on grasslands.

As a predator, *E. argus* could play a certain role in grasshopper control. *E. argus* is found on the Korean Peninsula, Mongolia, China and Russia (Chen, 1991) and potentially has an ability to adapt to a range of environments (Huey, 1982; Hu et al., 1986). Firstly, we could increase the population quantity of *E. argus* through habitat management to create an environment favorable to the survival and population growth of the species. For example, in semi-desert grasslands, the bush *Caragana microphylla* provides shelter for *E. argus* (Vitt and Price, 1982), because the plant functions to provide shelter for reproduction and avoiding predators such as hawks, foxes etc. Conservation and replanting that increase the distribution of bushes such as *C. microphylla* to facilitate an increase in *E. argus* populations and could be valuable in achieving the goal of having densities that provide grasshopper control. In areas where environmental degradation has led to local extinction of *E. argus*, reintroduction of lizards from environmentally compatible regions could be also useful. In summary, this study suggests that *E. argus* can be an important regulator of grasshopper populations, and potentially a valuable tool in IPM to control grasshoppers. In particular, by combining beneficial species known to control grasshoppers, such as the parasite *Nosema locustae*, the fungus *Metarhizium anisopliae* (Sieglaff et al., 1998; Lomer et al., 2001), and other natural enemies, grasshopper populations could be sustainably controlled in semi-desert grasslands.

## Conflict of interest

We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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