Contents

Chinese abstract

前人針對蜘蛛身上鮮艷色彩功能的研究顯示結網型蜘蛛其鮮豔色彩對於其獵物捕食扮演著重要的角色，然而針對徘徊型蜘蛛體色功能的探討則非常少。越來越多的報導顯示夜行性動物像是鳥類與青蛙似乎會在晚上利用視覺訊號來溝通。而夜行性溪狡蛛（*Dolomedes raptor*）其成熟個體有明顯的雌雄二型性，成熟雌性在第一、二對步足的前端具有白色色斑；成熟的雄性則是在頭胸部上形成白色的環狀色斑。為探討此顏色訊號之功能，我首先利用視覺生理方法量化其體表光訊號；並利用與溪狡蛛體色相仿之色紙做成有白環(n = 35)及無白環的蜘蛛模型(n = 46) 並利用夜視攝影機進行監控，探討體色視覺訊號所扮演之角色。而色斑操控的結果顯示在不同模型處理中，具白環者有較高之獵物靠近率，但此訊號在求偶中扮演何種角色仍不清楚。為回答此問題，我首先在野外採集成熟雄性個體攜回實驗室進行體型大小、重量、色斑面積及色斑光譜特性等各式型值的測量，來探討是否野外之族群在體色訊號上具變異性且與體型具特定關係。我接著在野外採集未成熟的雌性與雄性個體於實驗室固定條件下單獨飼養至成熟，在拍照後利用 GIMP 2 進行型值測量與色斑面積的量化，並同時利用光譜儀量化色斑的光譜性質，然後進行操縱

色斑的求偶實驗。實驗組利用與體色相似的棕色顏料去除色斑訊號，而控制組則會把顏料塗在背甲的棕色部位上以避免氣味的影響，並利用夜視攝影機記錄。野外的調查結果顯示雄性色斑在環境中具有變異，從色斑操縱實驗結果顯示出雄蛛色斑的有無會顯著影響雌蛛交配的接受程度；而雄蛛營養操控實驗的結果顯示，在不同營養處理下雄蛛在第二性徵上的訊號會根據營養好壞而有所差異，並會影響雄蛛蛻皮時間的快慢。綜合以上結果，雄蛛鮮明的體色在其求偶時似乎可做為物種識別之訊號及品質高低之指標，顯示視覺訊號在夜行性節肢動物的求偶過程中亦扮演重要角色。

Abstract

Previous studies investigating the function of conspicuous body coloration in spiders focus on orb-weaving spiders and the results show that such signal plays an important role in attracting prey. So far, few studies had examined the role of body coloration of nocturnal wandering spiders. More and more studies show that nocturnal terrestrial animals such as anurans and birds will use visual signal to communicate. The nocturnal fishing spider *Dolomedes raptor* exhibits sexual color dimorphism. Female has white hairs on metatarsus of first two pairs of legs and male has white stripes on both sides of cephalothorax. To realize the function of conspicuous coloration in male *D. raptor,* I first measured the spectra of this spider's body coloration. I then manipulated the color signal of male spiders by using dummies constructed from papers with reflectance spectra similar to those of real spiders. The control dummies had white stripes while the experimental dummies did not and the responses of prey and female *D. raptor* to them were monitored by infrared video cameras. The results showed that in the field the control dummies attracted significantly more prey. However, the exact roles

played by this conspicuous signal in the courtship of these nocturnal spiders are still unclear. To answer this question, I first collected mature male *D. raptor* from the field and measured their body size, weight, white stripe area and reflectance spectra to see whether individuals varied in these properties. Then I collected juvenile male and female *D. raptor* from the field and raised them individually under the same feeding conditions. When they matured I measured the body weight and took pictures of males and used GIMP 2 software to quantify the area and a spectrometer to measure reflectance spectra of white stripes. Males were divided into experimental (white stripes covered by brown paint) and control (brown paint on brown carapace) groups and females' responses to them were determined by laboratory experiments. The results of field survey show that males in the field exhibited variations in their white stripes. In the laboratory experiment, whether males exhibited conspicuous white stripes or not significantly affected the acceptability of females. The results of nutrition manipulation experiments showed that males receiving high nutrition intake exhibited larger white stripes and matured faster. My results demonstrate that conspicuous body color of male *D. raptor* seems to serve as species recognition signal and male quality indicator. Therefore, visual signal also plays an important role in the courtship of nocturnal arthropods.

Introduction

There are many kinds of communicate signals used by different animals inhabiting different kinds of environments. Visual signal is the most commonly used way of communication by animals. Visual display is used by organisms ranging from insects to mammals (Vallin et al., 2005, Caro, 2009). Some birds or fishes are active during day time and rely heavily on vision to court mates (Velando et al., 2006, Kodric-Brown and Nicoletto, 2001). If animals rely on visual signal to communicate, the prerequisite is that they should exhibit an appropriate visual system. The light condition in the environment is another important factor when animals are obtaining visual information.

Not only vertebrates, in invertebrates such as spiders visual signals are also involved in foraging in day time. Many researchers investigated the body colorful signal of orb-weaving spider and found that visual signal is very important for some spiders to attract prey (Tso et al., 2006). For example, the body of diurnal orb web spiders *Nephila pilipes* exhibit bright yellow spots on abdomen and legs and they can help spiders attract dipteran and hymenopteran insets (Tso et al., 2002). Tso et al. (2004)

quantified how body colors of *N. pilipes* were viewed by hymenopteran insects by a color contrast approach and found that yellow body parts were conspicuous when viewed against vegetation background but the color signal of black body parts and that of the vegetation background could not be easily distinguished. At night, some spiders also use their body coloration to attract prey. In a field manipulative study, Tso et al. (2007) used paint to change chromatic property of bright body parts of the orchid spider *Leucauge magnifica* and found that such treatment significantly reduced nocturnal insect catching ability of this spider. Chuang et al. (2008) used a paint with reflectance spectrum similar to that of the brown body parts of the nocturnal orb web spider *Neoscona punctigera* to cover the conspicuous yellow abdominal spots of this spider. Such treatment effectively decreased the prey catching performance of *N. punctigera*. According to above studies we can understand that the conspicuous coloration on spider’s body is not just a decoration but can help the spiders enhance their opportunities to attract more prey then obtain more energy and nutrient.

To realize whether certain animals use visual signals to communicate, an important thing to know is that the receiver must have the ability to receive and comprehend the meaning of such signal. Most spiders, especially orb-weaving spiders, have poor eyesight. So it is unlikely that they used body coloration as a signal to communicate between conspecifics. But some hunting spiders like jumping spiders and wolf spiders have good color vision and use color signal during courtship (Uetz and Smith, 1999; Pruden and Uetz, 2004). Researchers studying reproductive behaviors of wolf and jumping spiders showed that females can estimate males' quality by observing males' behaviors or ornaments. Under sexual selection pressures, no matter in the context of male-male competition or female choice, in the mating system of non-web spiders many communication signals in visual, acoustic, chemical, vibration or tactile modes are used (Huber, 2005). Most studies of the function of the visual signal in non-web spiders focus on the diurnal wolf spiders and jumping spiders. Both type of spiders have a good vision than web spiders and they develop many behaviors such as visual and vibrational display (Parri et al., 2002; Hebets et al., 2006). For example, when male wolf spider is courting a female, he will perform the drumming display by tapping the substrate with the pedipalps to transmit the vibrating and acoustic signal to the female. The female spider can assess the body quality of the performer by duration of vibration signal made by the male wolf spider (Parriy, 2002). Some other species of wolf spiders exhibit sexual dimorphism. In such species the males have lots of specialization on legs when they mature. Some species have hair tufts on the forelegs, some species have dark forelegs which contrast dramatically to other body parts and the other species have elongated or swollen forelegs (Framenau and Hebets, 2007). These ornamentations provide visual attribute such as size or symmetry for females to assess the quality of males. These ornaments have been shown to significantly affect the capulatory time of males and fertilization rate of eggs (Uetz and Smith, 1999). Result of previous studies showed that the specialized and ornamented forelegs of wolf spiders are used as secondary sexual traits and these traits play an important role in male courting behavior (Parri et al., 2002; Hebets et al., 2006). In male wolf spiders with sexually dimorphic traits such as tufts on the forelegs researchers found that both visual and vibration signal play an important role in the courtship during the day (Hebets et al., 2006). Different mode of communication signal does not seem to be operated independently. Taylor et al. (2005) reported that in wolf spiders vibration signals can modify the visual signals.

It is well known that visual signal is an important communication mode during the day. Limited by all sorts of constrains such as analytical methodology and visual equipments, most of studies about communications using visual signals focus on diurnal animals. During the night the ambient light intensity is very low and the noise to signal ratio is much higher than during daytime. Will animals active during the night also use visual signals to communicate in courtship? So far, few researches had explored this subject and many of them are conducted on frogs. Some nocturnal tree frogs had been observed to use visual displays during male-male combat. When an intruder male *Phyllomedusa boliviana* frog appeared, the resident male would wave their feet or hands in combination with acoustic signals (Jansen and Köhler, 2008). Some nocturnal tree frogs not only provide the acoustic signals to female frogs but also offer visual signal during courtship (Gomez et al., 2009; Richardson et al., 2010). When *Hyla arborea* frogs begin to call they also advertise the color of their vocal sacs. Richardson et al. (2009) reported that pigments of the colorful vocal sac are composed by carotenoids. This substance is considered to play an important role in immune system and therefore the color of vocal sacs can reflect the body condition of male frogs. Some nocturnal birds are also reported to use visual signal to communicate with each other. The nocturnal eagle owl parents will decide which owlet to feed according to the white feathers on the mouth of the owlets (Penteriani et al., 2007), and the white feathers can reflect the immunomodulatory ability of owlets.

In arthropods, a previous study showed that a spider *Leucorchestris arenicola* (family Heteropodidae) living in the desert rely heavily on visual signal for homing after foraging at night. When they move a short distance, they will stop to collect enough photons to continue moving forward (Nørgaard et al., 2008). This study shows that nocturnal vision does exist in certain non-weaving spiders and plays an important role in their daily lives. While most wolf spiders are active during day time, fishing spiders of the family Pisauridae are nocturnal wandering spiders. In most species of fishing spiders male and female body colors are very similar. According to field observations some species of fishing spiders have sexual cannibalism (Johnson and Sih, 2005). In some species males will make a "nuptial gift" seen like the egg sac while they are courting the female spider (Itakura, 1998). The nuptial gift made by male fishing spiders were proposed to be used by females to evaluate male body condition and such object can enhance male copulation times and fertilization rate of eggs (Stålhandske, 2001). Some researchers proposed that nuptial gift in fishing spiders might represent a kind of sensory trap. Males seem to use such visual signal to exploit females' innate preference for objects exhibiting egg-sac-like color and shape to avoid being attacked by female fishing spiders during mating (Stålhandske, 2001; 2002).

Luo and Chen (2010) reported that *Dolomedes raptor* is a nocturnal fishing spider inhabiting low altitude streams in Taiwan. Their prey includes semi-aquatic insects, small fish, tadpole and moths flying near the streams. When they are attacked by predators they will dive into water for more than half an hour. *D. raptor* has dramatic sexual dimorphism. The mature female spiders have white spots on the tips of first and second pairs of legs while the mature male spiders have two white stripes along the margins of cephalothorax. As far as we know, most nocturnal wandering spiders' body coloration is duller than that of diurnal species. In this study, I wonder why the nocturnal *D. raptor* has such a conspicuous body color signal. Since the visual ability of these spiders is potentially good, is it possible that these nocturnal spiders use the body color signal during courtship in a way similar to what has been reported from their diurnal relative wolf spiders? To answer this question I first used a dummy approach to manipulate the color signals of *D. raptor* to realize whether the white stripes in this species is a sexually-selected trait. I also constructed laboratory mate choice and food manipulation experiments to determine whether female *D. raptor* used males' white stripes to determine whether to mate or not and to assess the quality of males. This study shows for the first time that a nocturnal arthropod uses visual signal during courtship and as a cue to assess the quality of males.

Materials and Methods

The study site and the spider

*Dolomedes rapto*r are nocturnal wandering spiders and they inhabit low altitude streams in Taiwan. Their prey includes semi-aquatic insects, small fish, tadpole and moths (T.S. Lin unpublished data). When they are attacked by the predators such as crabs, they can dive into the stream for half an hour. When *D. raptor* complete the final molt and mature, they exhibit sexual color dimorphism, which phenomenon is similar to that of wolf spiders. The body colorations of male and female *D. raptor* are quite different. Mature female spiders have white spots at the tip of first and second pairs of legs and the mature male spiders have two bright white stripes at the two margins of cephalothorax (Figure 1).

I investigated and collected fishing spiders at low altitude streams near Dongshi Forest Recreation Area in Dongshi, Taichung city, Taiwan (E120°52’03.96”, N24°17’06.78”) and Lien-Hwa-Chih Research Center (LHCRC) operated by Taiwan Forest Research Institute situated in Yu-Chi, Nantou County, Taiwan (E: 120°52’36”, N: 23°55’13”). The habitat is characterized by presence of some medium-sized rocks along the banks of calm streams with slow water flow and relatively closed canopy. In addition to natural habitats such as streams in primary forests with few artificial constructions and low human disturbance, *D. raptor* can also be found in artificial habitats such as ponds or gutters with stagnant water.

<u>Determining the mating season of *D. raptor*</u>

In order to realize the mating season of the *D. raptor* I surveyed a population of this spider at a stream near Dongshi Forest Recreation Area. The field survey was conducted once every two weeks during the night from March, 2009 to April, 2010 for a year. During each field trip a line transect approximately 50 meters was set up along the banks of the stream and number of *D. raptor* of different development stage and sex in the transect was recorded. Months with the highest number of mature males and females were regarded as the mating season of *D. raptor*.

<u>Constructing dummy resembling male *D. raptor*</u>

To realize whether the white stripes of male *D. raptor* exhibited any function at night, I first used a dummy approach it. Compared with using live spiders, using dummies had certain advantages. First, dummies can be directly fixed on the stones nearby the stream to be monitored by infra-red video cameras. If live spiders were used they would move everywhere and it would be extremely difficult to monitor them by video cameras. Another advantage of using dummies is that confounding factors such as potential olfactory cues of male spiders could be well controlled and the effectiveness of visual signals could be unambiguously determined. The color signals of dummies resembled *D. raptor* in size and color investigate whether male *D. raptor* with white stripes would attract female spiders or would exhibit some additional function such as attracting prey.

To make sure that the color signals of dummies and real spiders were similar I first collected a number of different kinds of brown and white color paper. Then I used a spectrometer (USB-4000, Ocean Optics, Inc., Dunedin, Florida) to measure the spectra of spider's body colorations and used such information to choose color papers. A total of three spiders were collected from the field to measure reflectance spectra. After the paper was determined I used the following design to construct dummies. For the first group I used brown color paper to make the spider body and the white color paper to make white stripes to be pasted on the cephalothorax region. In another group I used the same brown color paper to construct the stripes then pasted them on the cephalothorax region. In both dummy groups glue was used to paste the stripes so the potential chemical cue of glue could be well controlled (Figure 2).

Field experiment using dummies

The field experiment was conducted in a stream near Dongshi Forest Recreation Area (E120°52’03.96”, N24°17’06.78”). In this experiment, dummies resembling male *D. raptor* with and without white stripes were used (dummies with white stripes: n=35; dummies without white stripes: n=46). Infra-red video cameras (Sony Hi-8, Didtitals-8, HDD SR-100 and SR-62 video cameras) were used to monitor events such as prey attraction, female attraction and predator attraction from 8: 00 PM to 4:00 AM. The field experiments were conducted between August 28 and September 1, 2009 and between July 17 and 24, 2010. In the field male *D. raptor* were frequently seen to sit on rocks along the stream. Therefore, I randomly chose rocks of appropriate size and placed two types of dummies on them alternately. During the field experiment each day 15 video cameras were used to monitor the dummies. They were placed about 1 m away from the dummy and the area monitored was about 0.25 m^2. After the field experiments were completed while viewing the video tapes in the laboratory the number of prey attraction events, female attraction events and predator attack events on dummies was recorded. These data was used to calculated attraction rates (number of events per hour of monitoring). Negative binominal regressions were used to compare the prey attraction rates of two types of dummies and logistic regressions were used to compare the female attraction rates and predator attraction rates of two types of dummies.

The effect of white stripes on female mate choice

I collected subadult males and females from the field in the spring and summer of 2011. All the spiders were raised in the lab under identical conditions until maturation to make sure that the spiders used in this experiment were all virgins. Each spider was individually raised in a plexigrass container (30 cm x 15 cm x 18 cm). The bottom of the container was submerged with 2 cm high of water and a sponge was placed for spider to perch. The water of the container was changed once every week. Opaque plastic sheets were placed between containers to prevent visual contact between individual spiders. I used crickets (*Achfa bimaculata*) with body length exceeding 2.0 cm to feed the female fishing spiders and those with body length between 0.5 to 1.0 cm to feed the male spiders. All the spiders were fed one crickets per week. When they matured, I used a digital waterproof camera (Olympus μ1030 SW) to take the picture of the spider and use GIMP2 software (version 2.6.11) to measure the foreleg length, body length, and the white stripes area on cephalothorax. I also weighed every individual before the experiment.

In this experiment I had two treatments. In the first treatment I used the brown paint matching the brown body color of *D. raptor* to paint the white stripes. Another treatment was the control and I applied the same quantity of brown paint on brown part of the cephalothorax. Spiders in both treatments were painted to avoid the potential influence generated by the chemical signal of paint. All of the spiders were used only once to make sure that their behaviors would not be influenced by prior experiences.

In order to perform mate choice experiments, I made a 30 x 30 x 15 cm arena then placed a cylindrical stage and plastic board at the bottom. The arena was covered by a piece of glass to prevent the spiders from escaping arena during the experiment. In each trial, I first introduced to the arena a mature female spider which molted two to three weeks ago and allowed her one hour to produce the drag line silks and release pheromones. Then, I would introduce a mature male *D. raptor* to the arena. In each trial three infra-red video cameras were used to record the spider's behaviors and interactions. The two infra-red video cameras (Sony SR-62 or SR-100 Series cameras) were mounted on both sides and another one was placed at the top to monitor the whole arena. Courting success was determined by whether the male could climb on female's back without being eaten. A chi-square goodness-of-fit test was used to compare the courting success of male *D. raptor* receiving different treatments.

Stripes color variation of male *D. raptor* in the field

Next, I would like to know whether the white stripes are an indicator of male quality, or it is just a species identification signal. If such signal represents a quality indicator I predict that the visual properties of such trait should vary and correlate with males' body conditions. I collected adult male spiders from the field in the spring and summer of 2011. All the spiders were brought to the laboratory and for each spider I took a photo by a digital camera (Olympus μ1030 SW) and measured the foreleg length, body length, cephalothorax width and white stripes area to quantify the variation pattern of spiders in the field. I also used a USB-4000 spectrometer to measure the spectrum of male's white stripe, and then used Avicol program (version 6, Gomez, D., 2006) to calculate the relative brightness. After the measurements all the individuals were brought back to the same stream but different collecting site to avoid sampling the same individual more than once.

Effect of nutrition treatments on signal property

In this part of experiment I tested whether male *D. raptor* receiving different level of nutrient intake would differ in brightness of white stripes. I collected juvenile male *D. raptor* from the field and raised them to sub-adult stage in the spring and summer of 2011 and 2012. Then I performed a pretest by measuring the reflectance spectra and area of the stripes. Then two nutrition treatments were performed. In the high nutrition treatment male spiders were fed one small cricket (*Achfa bimaculata*) with a body length of 0.5 cm every two days. In the low nutrition treatment spiders were fed same-sized crickets once every seven to ten days. When the spiders matured I measured the reflectance spectra and area of the stripes. Student *t*-tests were used to compare the relative brightness and area of white stripes of male *D. raptor* in the two groups before and after the nutrition treatments.

Results

<u>The mating season of *D. raptor*</u>

From the field observation, I discovered three phenotypes of female *D. raptor*. Most female only has white spots on the tips of first and second pairs of forelegs. A small number of females not only has white spots on the tips of the first and second pairs of forelegs, but also has faint white stripes at the cephalothorax. Finally, in some females their body and legs are covered with lots of gray broken patchy spots. The results of field survey showed that mature female spiders and juveniles where present all year round but mature male spiders only appeared from February to August in a year. Male spiders' temporal abundance pattern indicated that spring and summer are the major mating season of *D. raptor* (Figure 3).

<u>Paper used for constructing dummies</u>

After measuring the reflectance spectra of body coloration of spiders and those of hundreds of brown and white color papers I determined the paper for constructing dummies. The reflectance spectra of brown and white paper used were similar to that of spiders and were shown in figure 4 and 5.

Field experiment using dummies

While viewing the video footages in the laboratory I calculated prey, female and predator attraction rates and identified prey to taxonomic order. Results of field experiments showed that dummies with white stripes (control group, N=35) could attract more prey than the dummies without white stripes (experimental group, N=46) (Table 1, Figure 6). Results of a negative binominal regression showed that such difference was statistically significant ($z=3.936$, $p<0.001$). From the video footages I also found that dummies could successfully attract female *D. raptor* to approach them. The female attraction rate was not significantly different between dummies with white stripes or not (Figure 7). Results of logistic regression showed that the difference was not statistically significant (Table 2). From the video footages I also found that in addition to prey and females, dummies also attracted predators such as crabs. However, the predator attraction rates of two dummy types were similar (Figure 8) and results of logistic regression showed that they did not differed significantly (Table 3).

Effect of white stripes on female mate choice

Result of this part of study showed that white stripes seemed to affect their acceptance by female spiders during courting. Male *D. raptor* in the control group had white stripes and a total 15 trials were performed. The white stripes of males in the experimental group were covered by brown paint and a total of 14 trials were performed. Males in two groups did not differ significantly in body weight (*t*-test, t= 0.3659, *p*=0.7175). If a male had conspicuous white stripes, he usually would be accepted by females. Such males would spend some time weaving the forelegs and tapping the legs of females and females would eventually agree to let him approach her and climb on her back. However, when the male spiders' white stripes were covered by brown paint, when he started courting he would quickly be attacked and eaten by females. Males with white stripes had high probability of acceptance by females. Among 15 control group males used, only two of them were rejected by females. However, for experimental group males more than half of them were rejected and eaten by females (Figure 9). A Chi-square test showed that there was a significant difference in female acceptance between two groups (Chi-squared = 4.3654, df = 1, *p*-value = 0.03667).

<u>Stripe color variation of male *D. raptor* in the field</u>

In this part of study I would like to know if male *D. raptor* in the field exhibited variation in such visual trait, and whether such variation correlated with spiders' body conditions. I used USB-4000 spectrometer to measure the reflectance spectra of the spider's body coloration and calculated the brightness of white stripes of males collected from the field. The relative brightness of 33 mature male *D. raptor* collected from the field is given in figure 10. There was great variation in brightness of white stripes among males and stripe brightness was not correlated significantly with the body weight of spiders. The white stripes area of 33 mature male *D. raptor* collected from the field also varied considerable (Figure 11). The figure 12 showed the result of a linear regression showed that the total area of white stripes of male spiders collected from the field was positively correlated with male body weight (R^2=0.9009, p<0.001). Therefore, the visual signal of white stripes can potentially be used by females to assess quality of males.

Effect of feeding history on white stripes

To test the effect of foraging history on brightness of white stripes, I collected the juvenile males and raised them to subadult stage. Then I performed a pretest to measure the reflectance spectra of the stripes. The result (Figure 13) showed that the relative brightness of two group of spiders before the nutrition manipulation did not differ significantly (t-test, t = -0.2452, df = 24.438, p=0.8084). I then measured the reflectance spectra of the stripes again when they received nutrition treatments and matured. The results showed that the white stripe brightness of males receiving high nutrition treatment (n=19) and that of those receiving low nutrition treatments (n=14) (Figure 13) were not significantly differet (t-test, t = -0.5304, df = 25.807, p=0.6004). Result of a t-test showed that the total white stripe area of two group of spiders before the nutrition manipulation did not differ significantly (*t*-test, t = 0.4991, df = 28.209, p=0.6216). However, male *D. raptor* receiving high nutrition treatment (n=19) and those receiving low nutrition treatments (n=14) (Figure 14) differed significantly in the total area of white stripes (*t* -test, t = -2.9151, df = 30.041, p = 0.00666). The nutrition treatment not only affected the white stripes visual signal but also affected the molting time of male spiders (Figure 15). The male *D. raptor* receiving high nutrition treatment molted significantly faster than those receiving low nutrition treatment (*t*-test, t = -6.3602, df = 13.977, p<0.0001).

Discussion

So far, few studies had used fishing spiders of the family Pisauridae as the model organism to study biological or ecological issues. Because the fishing spiders are found in special habitats near the streams, most studies focus on these spiders' foraging strategies (Figiel and Miller, 1994), how they discriminate various stimuli, detect the waves generated by prey (Bleckamann and Lotz, 1987; Bleckamann et al., 1994; Kreiter and Wise, 1996) and how they move on the water surface (Gorb and Barth, 1994; Suter, 1999). In fishing spiders' natural habitats they will easily be attacked by invertebrate predators such as wasps (Roble, 1985) and vertebrate predators such as frogs. Therefore, fishing spiders' movement patterns in responding to attacks launched by frog predators were also examined (Suter, 2003). Interestingly, they have different movement patterns to cope with the survival needs of different periods (Kreiter and Wise, 1996). In this study I begin with the most basic investigation to understand the temporal abundance pattern of *D. raptor*. Results of the survey conducted for a year at Dong shi showed that adult female spiders and juveniles were present all year round but mature male spiders only appeared from February to August. Such pattern indicates that the mating season of *D. raptor* occurs in spring and summer. Since adult male spiders only appeared during few months, how to obtain a mate during this short period of time represents a strong selection pressure on them.

Results of this study demonstrated that the conspicuous body color of male *D. raptor* serves as species recognition signal and perhaps male quality indicator during courtship at night. In the dummy experiment, dummies with the white stripes could attract significantly more prey than the dummies without white stripes. Therefore, natural selection pressure of visually attracting prey to approach should be one driving force of evolution of white stripes in male *D. raptor*.

In the field, there is great variation in brightness and total area of white stripes among individual *D. raptor* males. The female spiders in the field seem to have ample opportunities to encounter males exhibiting different condition of white stripes. In my nutrition experiment, different nutrition treatment generated difference in the total area of male white stripes. In addition, the result of nutrition experiment also showed that different nutrition treatment also affected male molting time. Males receiving high nutrition treatment would molt faster than those receiving low nutrition treatment. If male *D. raptor* can molt earlier than other males, they can have more opportunities to find and court the female spiders. These results indicate that white stripes can be used by female to assess quality as well as species identity of males because whether the males have the white stripes or not will affect their acceptance by female. Since the white stripes on body of males are involved in courtship, such trait is also shaped by sexual selection pressures. Results of field dummy and laboratory female choice experiments indicate that the conspicuous white stripes of male *D. raptor* is under both natural and sexual selection, and it can enhance males' foraging success and female acceptance at night.

The conspicuous white stripes of male *D. raptor* may have multiple and complementary benefits. Such trait can help males attract more prey and render males higher nutrient intake to be able to molt faster or to display their good body condition to females. If this is the case, the selection pressures should generate bigger and bigger white stripes on the cephalothorax of males. So, what are the costs associated with such trait? The study by Fan et al. (2009) on factors shaping the visual lure design of giant wood spider *Nephila piplies* might provide some hint. The body coloration pattern of *N. pilipes* is composed of bright yellow spots embedded on black body part. The results of field experiment conducted by Fan et al. (2009) showed that such body coloration pattern reflected a trade off between opposing pressures of attracting prey and avoiding predators. They used a dummy approach to create *N. pilipes* of three body coloration patterns: those mimicking the black-and-yellow coloration pattern of female *N. pilipes*, those that were totally yellow and those that were totally black (Fan et al., 2009). They used video cameras to monitor responses of insects to these dummies in the field. The results of field experiments showed that although the yellow dummies could attract the highest number of prey than the standard and black dummies, the yellow dummies also attracted the highest number of predators. Such finding indicated that spiders with yellow signal greatly enhanced would enjoy great foraging benefit. However, such spiders would also suffer a severe cost of high predation pressure. Therefore, although current body coloration pattern of *N. pilipes* is not a best form of prey visual lure, it reflects a trade off between benefits and costs associated with bright coloration and is an optimal design. Not only diurnal orb-weaving spiders will use visual signal to lure prey, the nocturnal orb-weaving spiders such as *Neoscona punctigera* also attract nocturnal prey with the ventral yellow spots (Chuang et al., 2008). The size of these conspicuous color spots seem to also be limited by predation pressures from predators such as wasps (Chuang et al., 2008). During daytime, *N. punctigera* usually will perch on bark with their ventrum color spots well concealed to reduce the chance of being detected and attacked by predators. In my study, the white stripes on male *D. raptor* render the spiders multiple benefits such as attracting more prey and making them more acceptable to females. Judging from my nocturnal video recording on various dummies there seemed to be little predation cost associated with males with conspicuous white stripes. During night time dummies with white stripes did not attract more predators than those without white stripes. However, during day time the white stripes may render males some predation cost. Although male *D. raptor* usually hides between the stones during daytime, it seems to be prey of some parasitoid insects or wasps. Individuals with greatly enlarged white stripes will easily be detected and attacked by visual predators such as wasp during day time. It will be interesting to conduct the dummy experiment during day time to see whether white stripes will attract visual predators and generate predation cost to male *D. raptor*.

Sexual selection theories are often used to answer the question of why males and females are so different, and to explain the evolution of exaggerated male sexual traits. Female and male will use various kinds of communication channels during different stages of female choice and male-male competition (Huber, 2005). The secondary sexual traits in wolf spiders are very common. The male wolf spiders in different species have different ornamentations such as the elongation of the first pair of forelegs, the swollen of the tibia of forelegs or exaggerated pigmentation or brushes on forelegs (Framenau and Hebets, 2007). These visual traits play important roles in the process of female choice. Female wolf spiders could use the ornamentations to assess the body conditions of males (Rypstra et al., 2003; Pruden and Uetz, 2004; Taylor et al., 2005; Framenau and Hebets, 2007; Shamble et al., 2009). Some female wolf spiders evaluate the symmetry of the tufts on forelegs of male spiders to estimate male quality (Uetz and Smith, 1999). In the case of fishing spiders *D. raptor*, similar to the findings reported from wolf spiders they also have sexual dimorphism. The white stripes can render males higher probability of being accepted by female spiders and females could potentially use the total area of white stripes to estimate the male’s body conditions to decide whether to accept and mate with the male spider.

Results of field survey showed that male *D. raptor* in their natural habitats exhibited variation in a sexual visual trait. There is great variation in brightness and total area of white stripes among individual male spiders. Concluding from the results of laboratory feeding experiments and field survey, adult males of *D. raptor* varied in white stripe total area and molting time. At the beginning of mating season, the male spiders found in the field were usually larger than those emerged during later period. According to the results of feeding experiments, males receiving high nutrition treatment will obtain bigger white stripes and molted faster than those receiving low nutrition treatments. Variations in the visual sexual trait and molting time due to different nutriment intake should also occur in wild *D. raptor* populations. No matter the female matured or not they would have almost same opportunities to meet male *D. raptor* with different conditions in the field.

In wolf spiders, if the female spiders had courting experience before maturation they would have preference for males with ornamentation. On the other hand, the unexposed females did not exhibit preference for male spiders with the ornamentations (Hebets and Vink, 2007). It will be interesting to see whether such phenomenon also occurs in the nocturnal *D. raptor*. If the effect of courting experience on evolution of secondary sexual traits does exist in the *D. raptor* system it may explain why variations in male white stripes are present and how variations are maintained in the field.

Most visual communication researches on nocturnal animals focus on vertebrates. For example, the nocturnal eagle owl parents will use the white feathers on the mouth of the owlets to decide which owlets to feed because the white feathers could reflect the immunomodulatory ability of owlets (Penteriani et al., 2007). In anurans, more and more nocturnal tree frogs have been observed to use visual displays during male-male combat. When intruder *Phyllomedusa boliviana* male frog appeared, the resident male would weave his feet or hands in combination with acoustic signal (Jansen and Köhler, 2008). Some nocturnal tree frogs such as *Hyla* *arborea* not only provide acoustic signal to female frogs but also offer visual signals during courtship (Jansen and Köhler, 2008; Gomez et al., 2009; Richardson et al., 2010). When the *Hyla arborea* frogs start calling at night, they also advertise the color of vocal sac. Richardson et al. (2009) reported that pigments of colorful vocal sac are mostly carotenoid (Richardson et al., 2009). This substance is considered to be closely associated with operations of immune systems, so male colorful vocal sac can reflect male body condition in the courting process. Many studies investigating the courtship behavior found that communication signals cannot be easily separated and many channels are operating in the same time (Gomez et al., 2009; Richardson et al., 2010). Many animals court for mates at night use multimodal communicating signals. For example, in some tree frogs although the visual signal of vocal sac color plays an important role at night and in females use such signal to assess the male body quality, such display must be accompanied with acoustic signal to initiate females' response (Gomez et al., 2009; Richardson et al., 2010). In my experiment, it is demonstrated for the first time that visual signal alone is sufficient to signal a male's species identity and to indicate male body conditions to female *D. raptor* during courting at night. The courtship process of male *D. raptor* includes the following four steps: (1) waving forelegs (2) following female's drag line (3) tapping female's legs and (4) climbing to female's back. Visual signal seems to play an important role on the first three steps. Female spider seems to estimate male's body condition directly by visual signal. In the last two steps (3and 4) both tactile and vibrating signals were forwarded to female. So during different steps of courting female *D. raptor* seems to use multiple signals to estimate the male body quality. Previous studies also showed that in *Schizocosa* wolf spiders when male spiders were weaving their ornamented forelegs both visual and the seismic signals were transmitted to females during courting (Taylor et al., 2005; Hebets et al., 2006).

Fishing spiders have been reported to exhibite sexual cannibalism (Johnson, 2005; Johnson et al., 2005). Some Pisauridae males present a nuptial gift to female spiders after being touched by the females. If there was no nuptial gift males could not initiate response from the females (Itakura, 1998). The size of nuptial gift is correlated with the duration of copulation and proportion of eggs fertilized (Stalhandske, 2001). Because females have an innate behavior of protecting the egg sacs, so

Stalhandske (2001 and 2002) proposed that nuptial gift represented an exploitation of female's parental behavior. Previous studies show that fishing spiders can use visual signal to communicate and they can recognize the bright and dark colors. The nuptial gift built by some Pisauridae males looks like the egg sac made by females. Such resemblance in color may reduce female sexual cannibalism and consequently increase sperm transfer time and egg fertilization rate (Stalhandske, 2001, 2002; Johnson, 2005). My results show that conspicuous body color of male *D. raptor* serves as a species recognition signal and perhaps male quality indicator. The conspicuous color of male spiders can reduce the risk of being eaten by females during courting. There was variation in the total area of white stripes on adult male *D. raptor* in the field and such variation seemed to result from these spiders' foraging history. It will be interesting to investigate whether visual signal also plays important role in the courtship of other nocturnal animals in the future.

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Tables

Table 1. The result of negative binominal regression comparing prey attraction rates of male *D. raptor* dummies with white stripes (WM) and dummies without white stripes (BM).

Parameter	Group	Estimate of β	SE	z	*p*
Intercept		-2.0505	0.2005	-10.229	<0.0001
Treatment	WM	1.0592	0.2691	3.936	<0.0001
Treatment	BM	0.0000	0.0000		

* The β of BM treatment group was arbitrarily designated as 0 to facilitate comparisons of probabilities of different events.

Table 2. The result of logistic regression comparing female spider attraction rates of male *D. raptor* dummies with white stripes (WM) and dummies without white stripes (BM).

Parameter	Group	Coefficient	SE	Wald z	p
Intercept		-3.43063	2.5674	-1.34	0.1815
Treatment	WM	1.06815	0.9156	1.17	0.2434
Time		0.04976	0.3591	0.14	0.8898

Table 3. The result of logistic regression comparing predator attraction rates of male *D. raptor* dummies with white stripes (WM) and dummies without white stripes (BM).

Parameter	Group	Coefficient	SE	Wald z	p
Intercept		-8.2980	3.9581	-2.10	0.0360
Treatment	WM	0.2588	0.9919	0.26	0.7942
Time		0.7783	0.5148	1.51	0.1306

Figures

(a)

(b)

Figure 1. Male (a) and female (b) *Dolomedes raptor* showing their dimorphic body coloration patterns.

(a) (b)

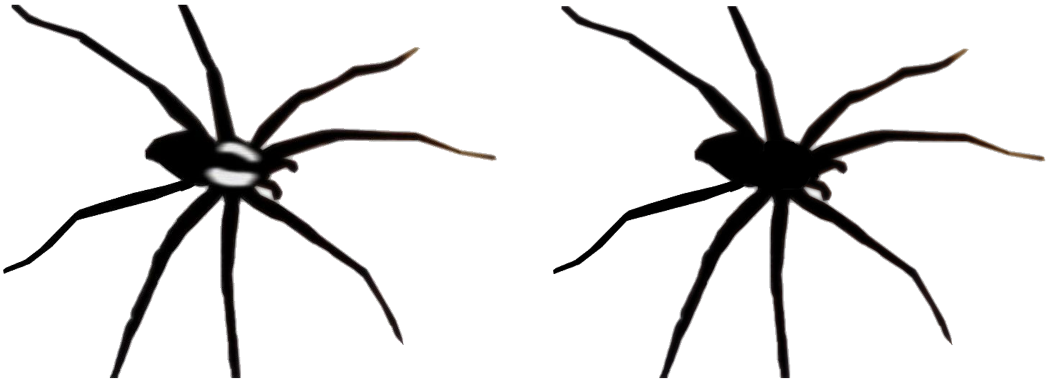

Figure 2. A schematic drawing of dummies used in (a) control (white stripes present) and (b) experimental (white stripes removed) groups.

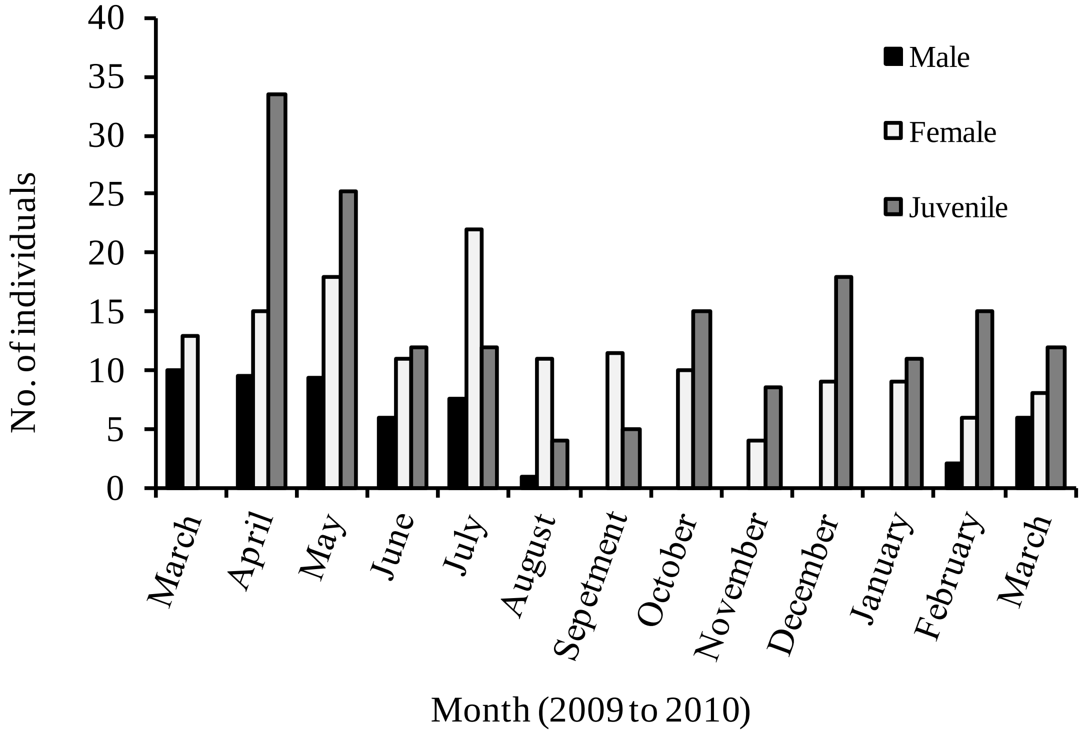

Figure 3. Temporal abundance pattern of a *D. raptor* population in Dongshi, Taichung city, Taiwan recorded from March, 2009 to April, 2010.

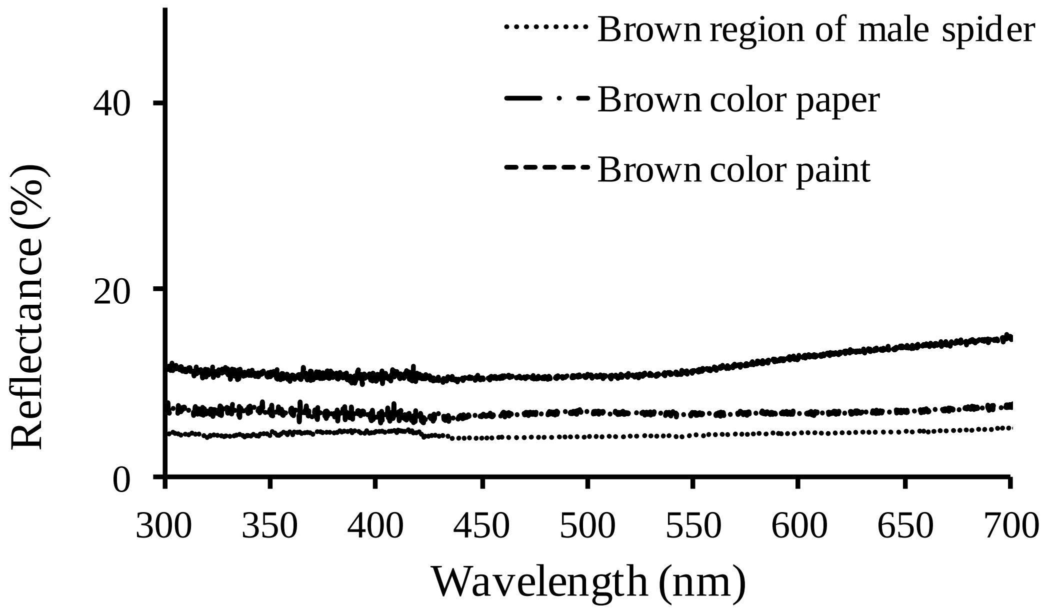

Figure 4. The reflectance spectra of brown region of male *D. raptor*, brown color paper used to construct dummies, brown paint used in mate choice experiment.

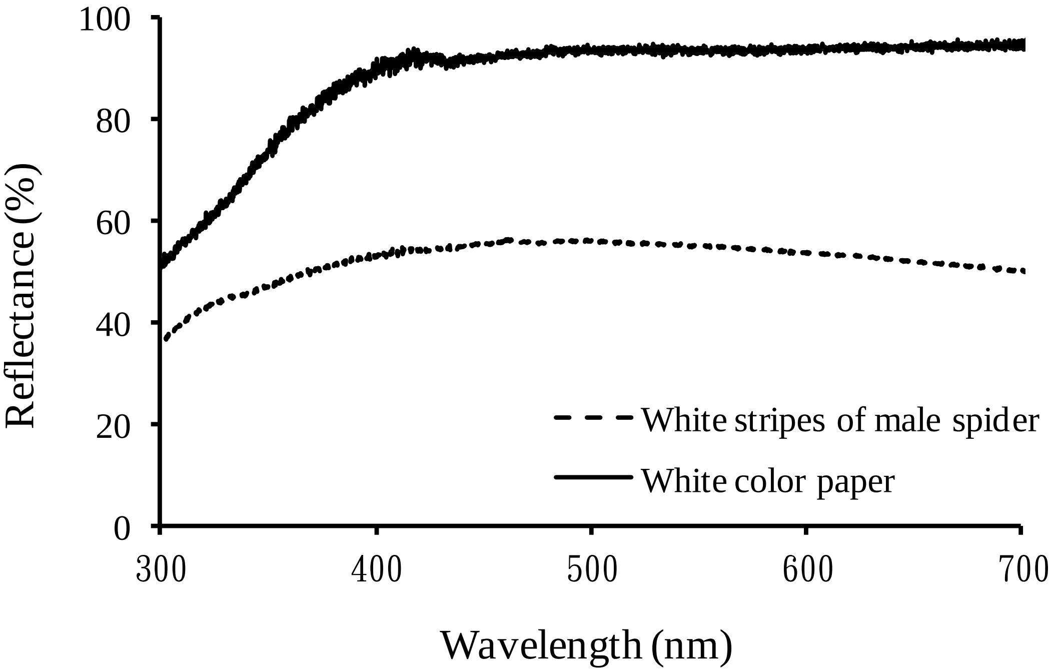

Figure 5. The reflectance spectra of white stripes of male *D. raptor* and white color paper used to construct dummies.

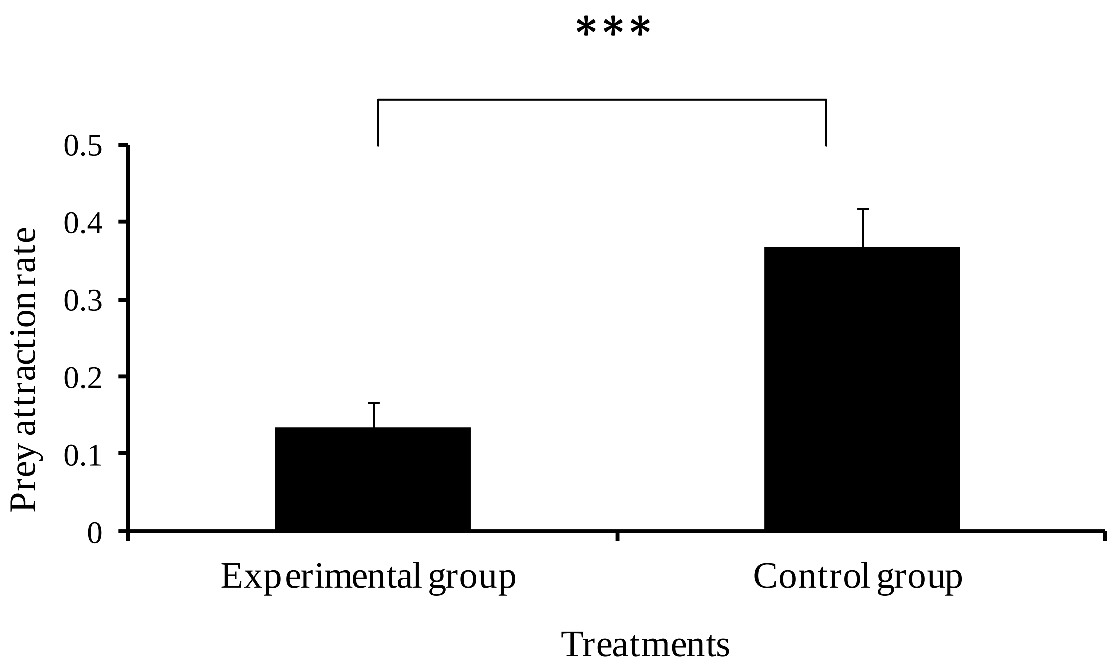

Figure 6. Mean (±SE) prey attraction rates (number of prey attracted per hour of monitoring) of dummies in control (white stripe present, n=35) and experimental (white stripe removed, n=46) groups. (***: P<0.001).

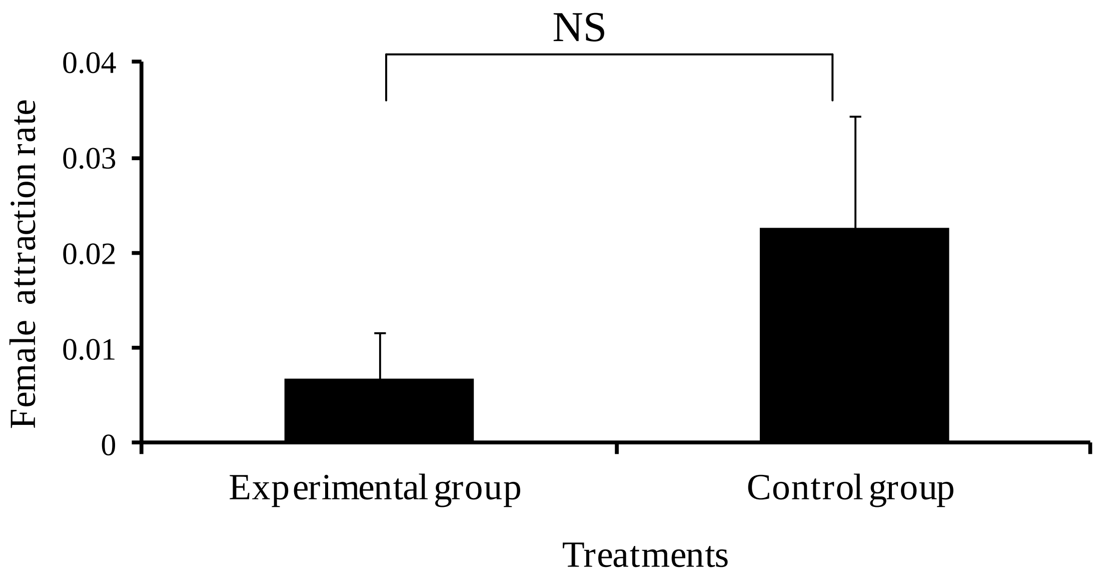

Figure 7. Mean (±SE) female attraction rates (number of female attracted per hour of monitoring) of dummies in control (white stripe present, n=35) and experimental (white stripe removed, n=46) groups. (NS: nonsignificant).

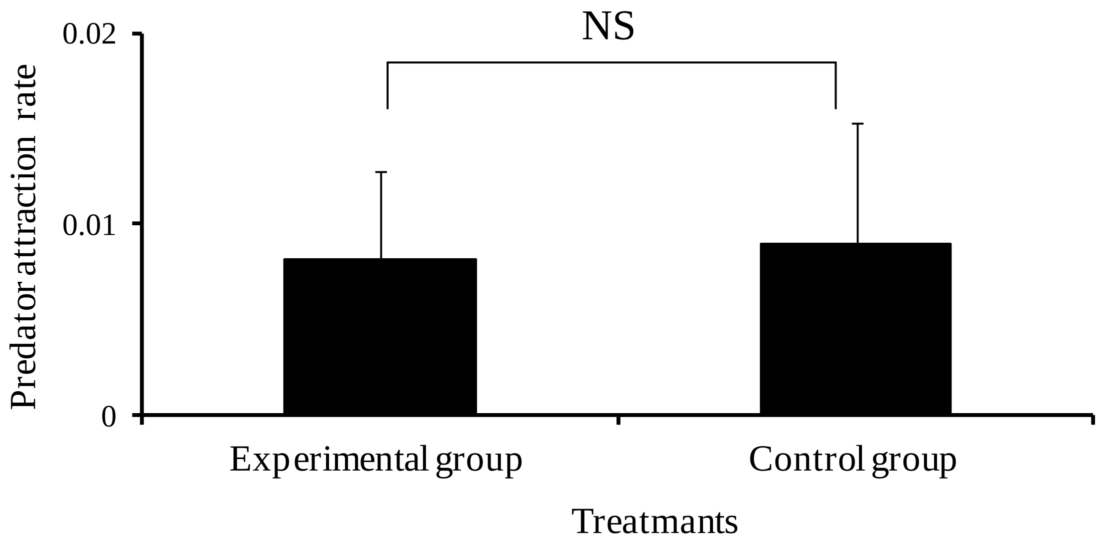

Figure 8. Mean (±SE) predator attraction rates (number of predator attracted per hour of monitoring) of dummies in control (white stripe present, n=35) and experimental (white stripe removed, n=46) groups. (NS: nonsignificant).

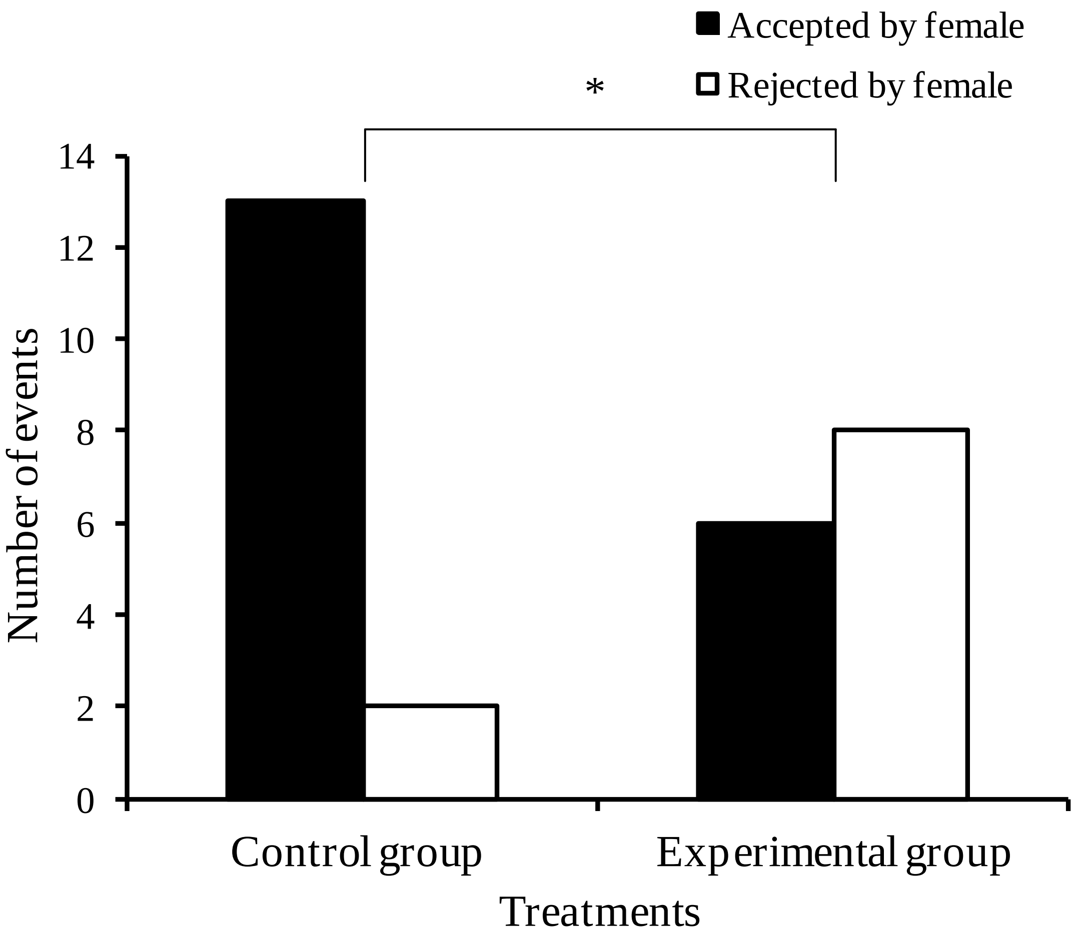

Figure 9. Courting success of male *D. raptor* in the experimental (white tripes covered by paint) and control (paint on brown part) groups. (*: P<0.05).

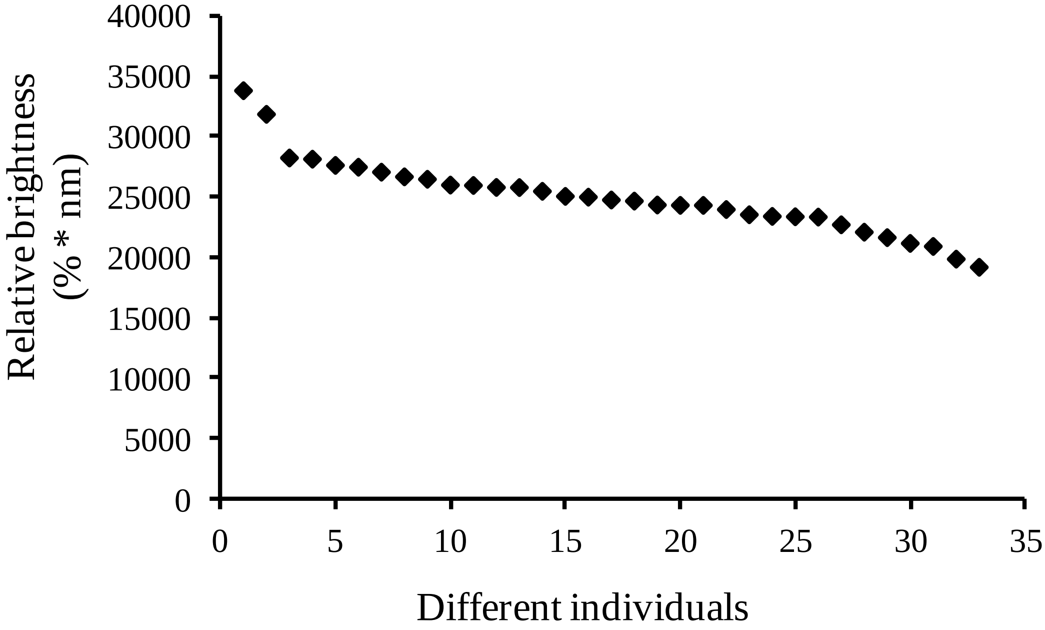

Figure 10. Relative brightness of white stripes of mature male *D. raptor* (n=33) collected from the field showing the extent of variation among individuals.

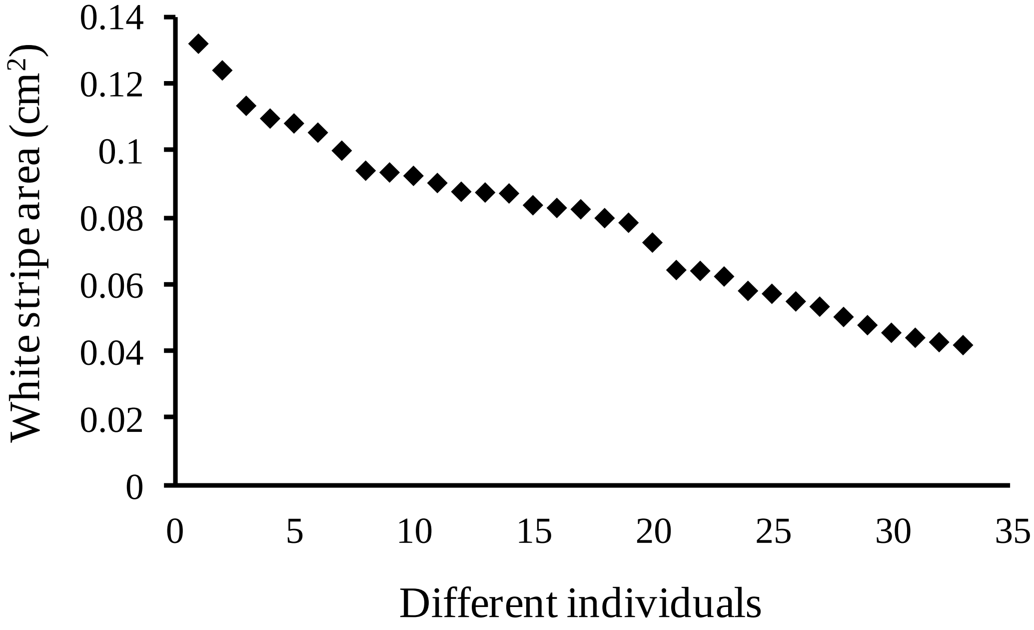

Figure 11. The total area of white stripes of mature male *D. raptor* (n=33) collected from the field showing the extent of variation among individuals.

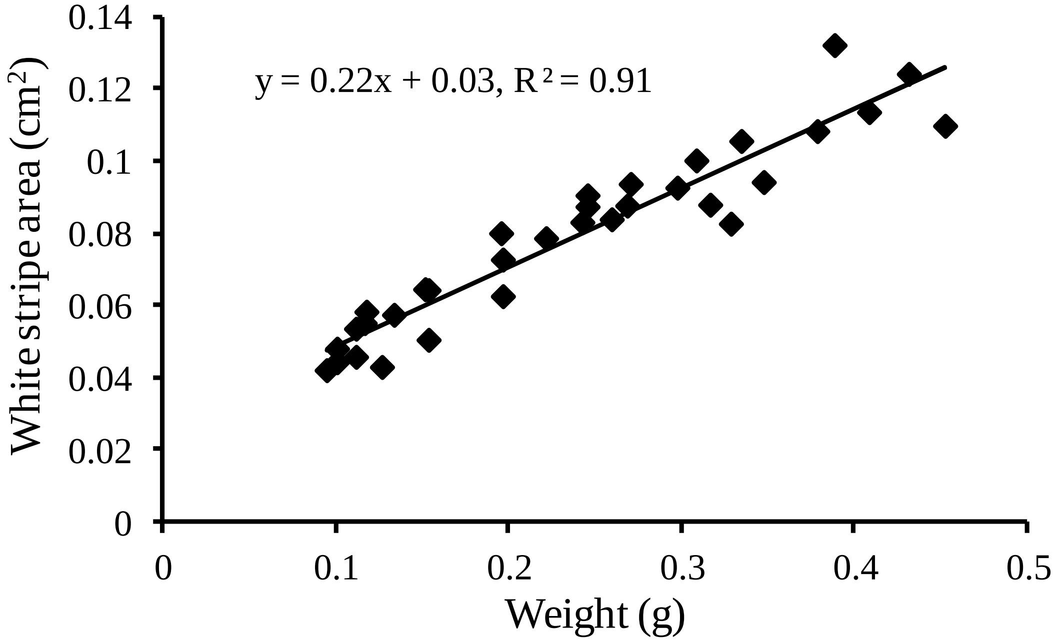

Figure 12. Relationship between total area of white stripes and body weight of mature male *D. raptor* (n=33) collected from the field.

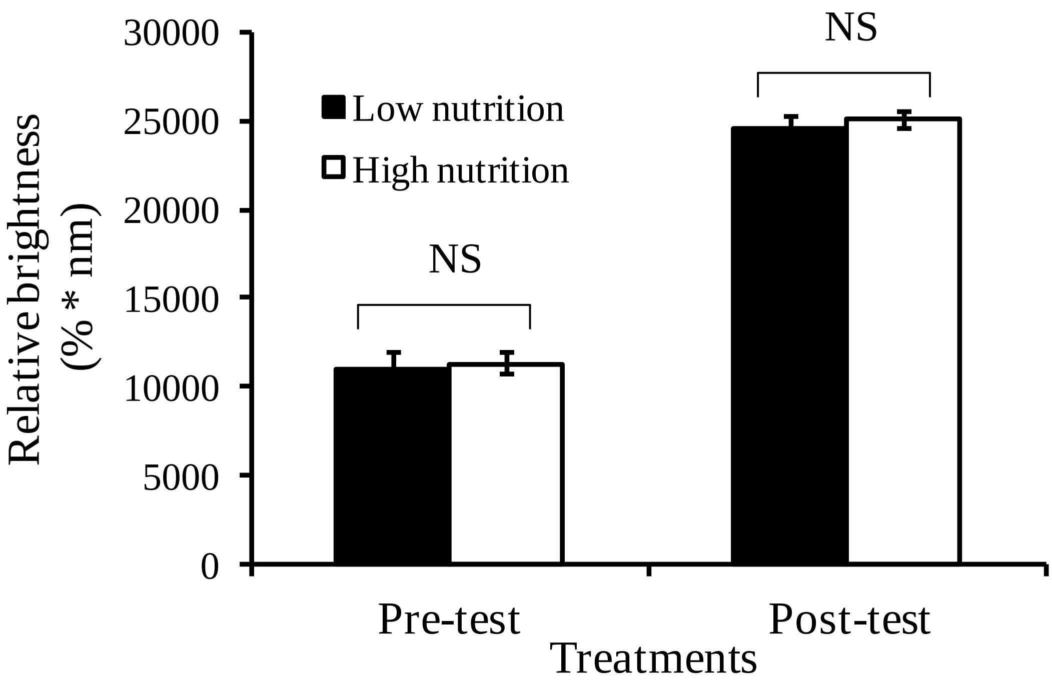

Figure 13. Mean (±SE) relative brightness of male *D. raptor* white stripes before (pre-test) and after (post-test) receiving low and high nutrition treatments. (NS: nonsignificant).

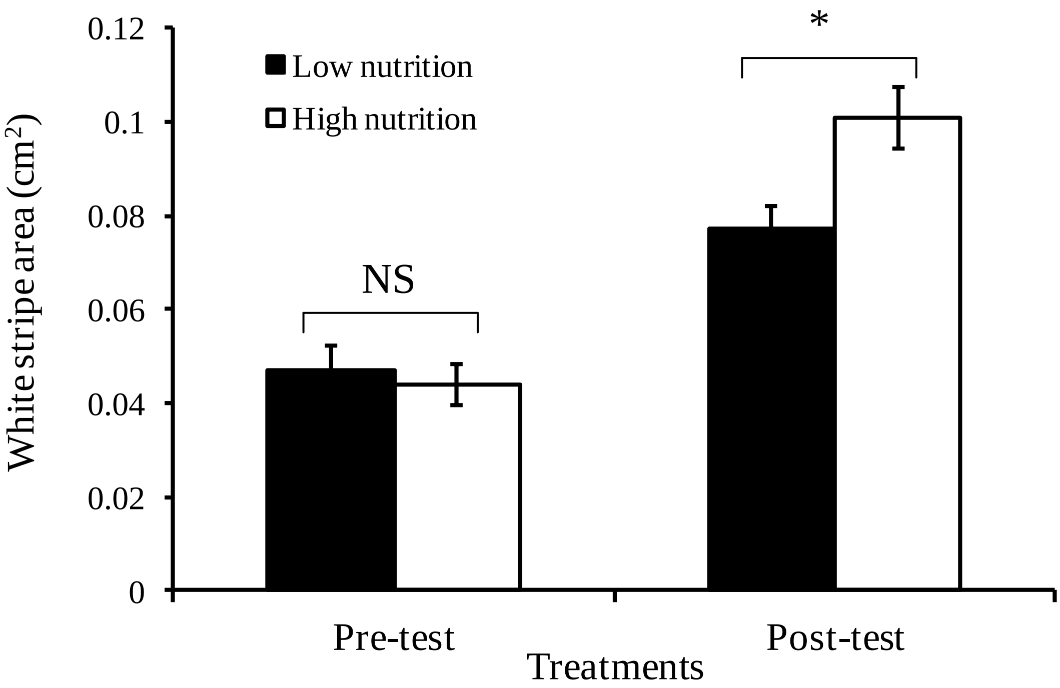

Figure 14. Mean (±SE) white stripes area of male *D. raptor* before (pre-test) and after (post-test) receiving low and high nutrition treatments. (NS: nonsignificant) (*: P<0.05).

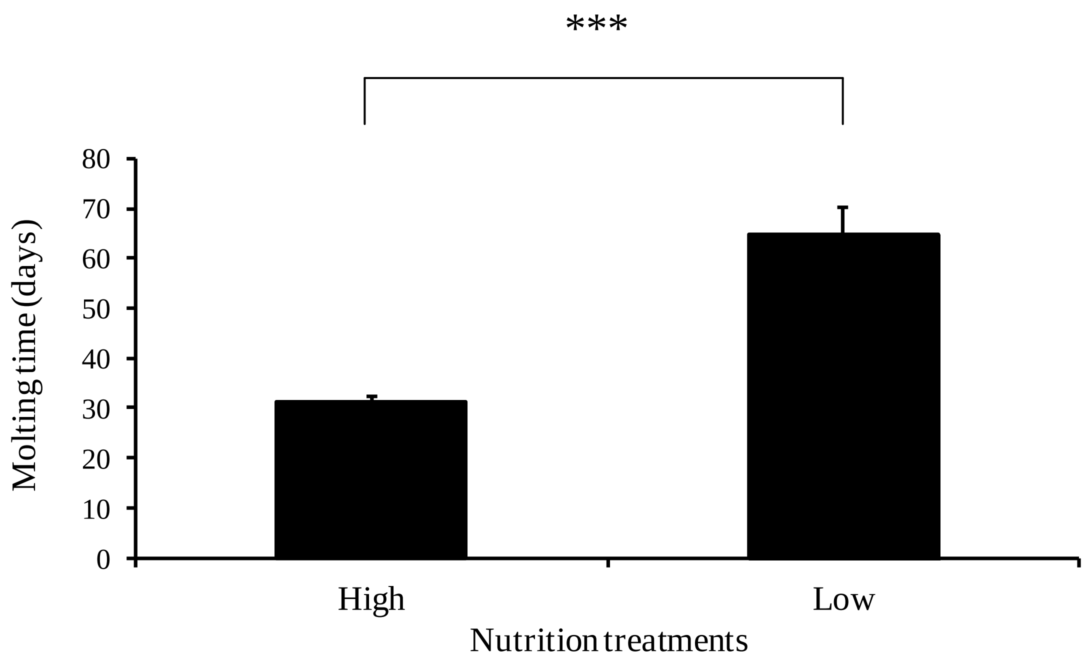

Figure 15. The molting time of male *D. raptor* receiving low and high nutrition treatments. (***: P<0.0001).