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ORIGINAL ARTICLE

Evolution of spiders from nocturnal to diurnal gave spider silks mechanical resistance against UV irradiation

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During their evolutionary history, some species of spiders have changed from a nocturnal to a diurnal lifestyle, and the important change in their environment was irradiation by sunlight. Orb webs of diurnal spiders may be markedly affected by exposure to ultraviolet (UV) irradiation, whereas those of nocturnal spiders may be unaffected. It is of great interest to consider the effects of UV rays on the mechanical properties of spider silks from the viewpoint of evolution. The webs of orb-weaving spiders must trap insect prey, which closely relates to the breaking energy of the spider silk constituting the orb webs. The effects of UV rays on the mechanical properties, particularly the mechanical breaking energy, of the silks of diurnal and nocturnal spiders were investigated. UV rays mechanically strengthened the draglines of diurnal *Nephila clavata* and two other kinds of diurnal spiders (*Leucauge blanda* and *Argiope bruennichii*), suggesting that the ability of the orb web to capture insects requires less maintenance. However, UV rays mechanically weakened the draglines of nocturnal *Yaginumia sia* and one other kind of nocturnal spider (*Neosona nautica*), suggesting a decrease in the ability of the orb web to capture insects. The results provide strong support that diurnal spiders are in a more evolved stage than nocturnal spiders, so they secrete silks with an evolved mechanical resistance against UV irradiation. This study suggests a means to search for biological materials with resistance to UV rays.
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INTRODUCTION

Ultraviolet (UV) rays are generally harmful to living organisms, including animals and humans,[1] as well as to specific biological materials, such as silkworm silk.[2–4] As a result, the mechanical functions of silks that constitute the orb webs of diurnal spiders for capturing insect prey outdoors could be expected to be markedly affected by exposure to UV irradiation. Because UV irradiation rapidly decomposes silkworm silk, it is very important to search for biological materials with resistance to UV rays, as the quantity of these rays has increased recently owing to ozone layer depletion.[5] However, there are no appropriate methods for searching for them.

In their evolutionary history, some species of spider have changed from nocturnal to diurnal creatures.[6,7] An important environmental feature of the diurnal lifestyle is irradiation by sunlight. The visible and UV reflectance of spider silks has been previously reported.[8–10] It is of great interest to study how spiders changed from nocturnal to diurnal habits in the evolutionary process from the viewpoint of the effects of UV rays on spider silks. Despite the potential importance of studying the effects of UV rays on spider silks, the implications concerning the evolution of a diurnal lifestyle have not yet been explored. In this study, the difference between nocturnal and diurnal habits of spiders is investigated with respect to the effects of UV rays on spider silks.

Naturally occurring UV rays from sunlight irradiate living creatures that are active not at night but during the daytime. The daylight performances of orb webs of diurnal spiders thus are markedly affected by UV irradiation. However, the orb webs of nocturnal spiders are not affected because no UV rays reach the earth at night. The webs of orb-weaving spiders must be able to trap insect prey. This ability is closely related to the breaking energy of the orb webs, which is dependent upon the mechanical breaking stress (BS) and mechanical breaking strain (BN) of the spider silk. If UV rays were to disrupt the mechanical function of orb webs, they can fail to effectively trap insects. Therefore, it would have been necessary for spiders to build orb webs that were resistant to UV rays to allow them to become active during the daytime. However, it is unknown whether spider silks have properties that protect them against UV rays.

Previously, the effects of UV rays, including UV-A (320–400 nm), UV-B (290–320 nm) and UV-C (200–290 nm) rays, on spider draglines were studied using electron spin resonance,[11,12] and it was shown that

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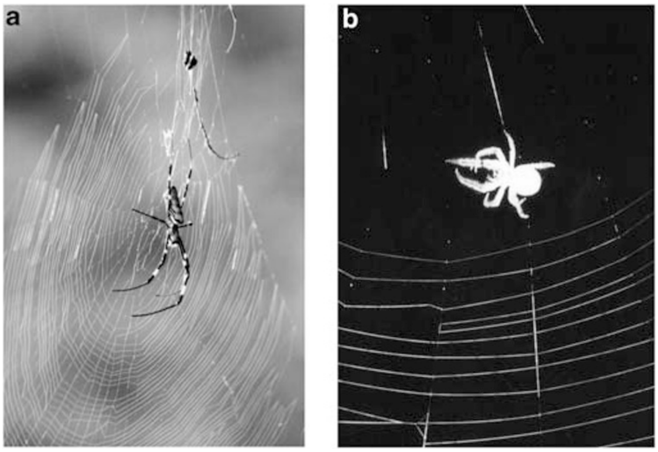

Figure 1 A diurnal *N. clavata* spider and a nocturnal *Y. sia* spider on orb webs. (**a**) A diurnal *N. clavata* spider waiting for insect prey on an orb web. (**b**) A nocturnal *Y. sia* spider rebuilding its orb web in the evening.

UV-A rays mechanically strengthened the draglines of diurnal *Nephila clavata* (L. Koch) spiders (Japanese golden web spiders) (Figure 1a),[13] although the impact on the mechanical breaking energy was not investigated. Determining whether the silk of nocturnal spiders is comparatively weaker or stronger in mechanical resistance to UV irradiation, with respect to the mechanical breaking energy, could help to clarify this stage in the evolutionary history of spiders.

To explore this issue, UV irradiation experiments were performed using UV rays with an intensity distribution similar to sunlight. A difference in the resistance to UV rays between the silks of diurnal and nocturnal spiders would elucidate the evolutionary history of spiders. It is important to know whether UV rays maintain, weaken or strengthen the mechanical function of silks secreted from diurnal and nocturnal spiders. Such knowledge will provide an important way to search for biological materials with resistance to UV rays.

This study describes findings regarding the mechanical resistance to UV rays of the silks of nocturnal and diurnal spiders, provides evidence to support the evolution of spiders and also provides an important clue for obtaining spider silks with mechanical resistance to UV rays from an evolutionary standpoint.

MATERIALS AND METHODS

Sample preparation

The spiders used here were mainly diurnal *N. clavata* and nocturnal *Yaginumia sia* (STRAND) spiders. Diurnal *Leucauge blanda* (L. KOCH) and *Argiope bruennichii* (SCOPOLI), and nocturnal *Neosona nautica* (L. KOCH) spiders were also used for comparison.

Female diurnal *N. clavata* spiders, which are active in the daytime, rebuilt approximately half of their orb webs every night. In other words, both halves of the orb webs were rebuilt every 2 days. *L. blanda* and *A. bruennichii* spiders were also active on the orb web in the daytime outdoors. In contrast, nocturnal *Y. sia* spiders that rebuild their orb webs every evening were active on the webs only at night and retreated to a sheltered position every morning. Broken webs where there were no *Y. sia* spiders were usually observed in the daytime. *N. nautica* spiders were active at night and often stayed on their orb webs even in the daytime.

Orb webs are mechanically supported by a main frame of radial threads, which are similar to the draglines that act as 'mechanical lifelines' for falling spiders by supporting their body weight.[14,15] Although the radial threads should be used to study the effects of UV rays on the orb webs, it is not appropriate to use the radial threads cut directly from the built orb web as samples because of the mechanical hysteresis associated with cutting and building. Thus, it is appropriate to estimate the mechanical strength of the orb web using the draglines instead of the radial threads, because both types of threads are secreted from the same ampullate gland.[6]

In this study, samples of the draglines produced by diurnal *N. clavata*, *L. blanda* and *A. bruennichii*, and nocturnal *Y. sia* and *N. nautica* spiders were investigated. Live spiders just after being captured from the orb webs were used in the experiments. A spider was made to fall from a wooden bar under laboratory conditions to avoid the mechanical hysteresis of the draglines ascribed to the extra stress. Long draglines were obtained from the spider by sticking the draglines to both sides of a plastic frame that was $\sim$50 cm long and covered with an adhesive, and then the draglines were cut into eight specimens stuck to both sides of a paper frame covered with an adhesive for mechanical measurements. One specimen was designated for scanning electron microscope observation and the other specimens were used for UV irradiation for fixed periods. This sampling procedure was repeated more than five times for each live spider.

Scanning electron microscope (JEOL, Tokyo, Japan) images were used to ascertain whether the samples were true draglines consisting of double filaments and to determine the cross-section area of each dragline.

Mechanical measurements

The stress–strain curves of the silk draglines were carefully measured using a Tensilon UTM-IIIL instrument (Instron Japan, Tokyo, Japan) at a stretching velocity of $3.3\times10^{-4}\,\mathrm{m\,s^{-1}}$.[13,15] The 4-cm long dragline samples for UV irradiation were prepared by cutting the long dragline secreted from each spider. Mechanical measurements were repeated for five dragline samples irradiated by UV rays for the same amount of time, and an average value and a standard deviation were calculated. The BS and BN, defined, respectively, as the stress and strain at the breaking point, were determined from the stress–strain curves of the draglines. The mechanical breaking energy, which is defined as the work necessary to break the spider silk, of the orb webs depends on the BS and BN of the silk. The mechanical breaking energy can be roughly estimated as the BS multiplied by the BN, although it can be exactly determined from the integral area of the stress–strain curves of the spider silks.

UV rays

UV rays reaching the ground from the sun are mainly UV-A rays (320–400 nm). The intensity of the UV-B rays (290–320 nm) reaching the ground from the sun is very weak, and no UV-C rays (270–290 nm) reach the earth's surface. Thus, UV-A rays were the most appropriate radiation for these experiments. UV-A rays (hereafter referred to as UV-A* rays) were prepared by excluding the wavelengths shorter than 320 nm from a 660-W xenon-arc lamp (Suntester XF-180, Shimadzu, Japan) with a special filter, thereby providing an intensity distribution similar to sunlight. The intensity of the UV-A* rays was similar to that of natural UV-A rays. In the UV irradiation instrument, samples were set 25 cm away from the xenon-arc lamp and an air fan was installed to avoid overheating.

Electrification

The electrification of the draglines was measured after UV irradiation using a Faraday cage (KQ-1400; Kasuga Denki Inc., Tokyo, Japan).

RESULTS

Diurnal spiders

UV-A* irradiation changed the stress–strain behavior of draglines secreted by *N. clavata*, a diurnal spider, leading to a marked increase in the BS.[11] The time-dependent changes in the BS and BN values of the draglines of a diurnal *N. clavata* spider weighing 385 mg (Figure 1a) after UV-A* ($>$320 nm) irradiation are shown in Figures 2 and 3, respectively. Here, the normalized BS and BN used in Figures 2 and 3 were obtained by dividing the observed values of BS and BN by the initial values before UV irradiation. The BS increased rapidly shortly after UV-A* irradiation commenced, peaked relatively quickly and then decreased gradually as it approached an asymptotic value (see Figure 2). The UV-A* rays were found to mechanically strengthen the

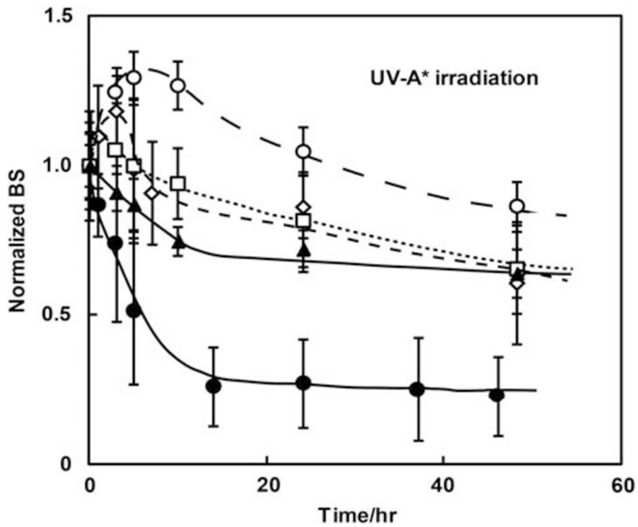

Figure 2 Time dependencies of the mechanical breaking stress (BS) of draglines secreted by a diurnal *N. clavata* spider weighing 385 mg and a nocturnal *Y. sia* spider weighing 153 mg. The draglines were irradiated with UV-A* rays, which were produced by removing wavelengths shorter than 320 nm from a xenon-arc lamp with special filters. ○: *N. clavata*, ●: *Y. sia*. For comparison, time dependences of the BS of draglines secreted by a diurnal *L. blanda* spider weighing 71 mg, a diurnal *A. bruennichii* spider weighing 601 mg and a nocturnal *N. nautical* spider weighing 203 mg are plotted. □: *L. blanda*, ◇: *A. bruennichii*, ▲, *N. nautical.* The values (BS) shown here were normalized by dividing the observed values of the breaking stress by the initial values (*N. clavata,* 1.20 GPa; *Y. sia,* 1.15 GPa; *L. blanda*, 1.30 GPa; *A. bruennichii*, 1.01 GPa; *N. nautica*, 1.47 GPa) .

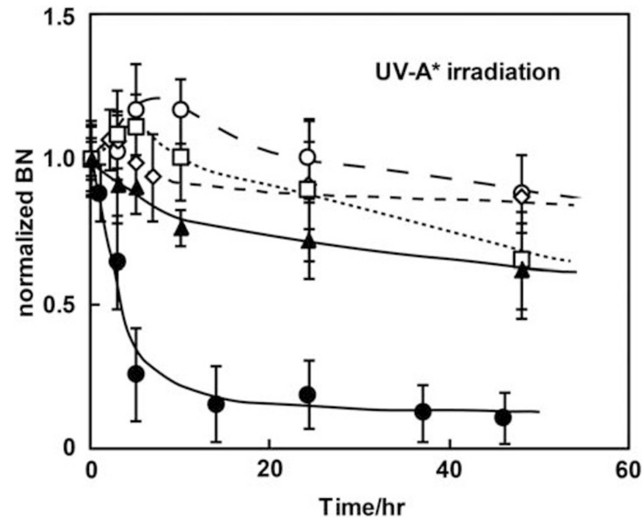

Figure 3 Time dependencies of the mechanical breaking strain (BN) of draglines secreted by a diurnal *N. clavata* spider and a nocturnal *Y. sia* spider. The draglines were irradiated with UV-A* rays, which were prepared by removing wavelengths shorter than 320 nm from a xenon-arc lamp with special filters. ○: *N. clavata*, ●: *Y. sia*. For comparison, the time dependences of the BS of draglines secreted by a diurnal *L. blanda* spider weighing 71 mg, a diurnal *A. bruennichii* spider weighing 601 mg and a nocturnal *N. nautical* spider weighing 203 mg are plotted. □: *L. blanda,* ◇: *A. bruennichii*, ▲: *N. nautical.* The values (BN) shown here were normalized by dividing the observed values of BN by the initial values (*N. clavata*, 24.1%; *Y. sia,* 23.2%; *L. blanda*, 20.9%; *A. bruennichii*, 21.9%; *N. nautica*, 24.9%).

draglines. The UV-A* rays caused the BS to remain elevated compared with the baseline value (that is, the start of the irradiation period) for ~28 h, and the peak level was ~35% higher than the baseline. However, UV-A* irradiation for >28 h, which corresponds to a sunlight-irradiation period of about 2 days, decreased the BS below the initial value, suggesting the destruction of the mechanical functions of the orb webs. The asymptotic value was lower than that at the baseline. In contrast, UV-A* irradiation had no effect on the BN of draglines secreted by a diurnal *N. clavata* spider, which remained relatively constant during the fixed period (Figure 3). The observed increase in the BS and the maintenance of the BN under UV-A* irradiation may be related to the mechanical strengthening of the orb webs in terms of the breaking energy.

UV-A* irradiation rapidly increased the BS values of the draglines of a diurnal *L. blanda* spider weighing 71 mg and a diurnal *A. bruennichii* spider weighing 601 mg. Small peaks in the time-dependent changes in the BS values appeared, and then the peaks gradually decreased. The peaks were relatively small compared with those for the *N. clavata* spider. Thus, UV-A* irradiation also mechanically strengthened the draglines of diurnal *L. blanda* and *A. bruennichii* spiders (Figures 2 and 3). This result suggests that the silks of diurnal spiders are strong or at least not weakened by UV-A rays, although all diurnal spiders were not examined.

UV rays, including UV-A and UV-B rays with wavelengths >290 nm (referred to as UV-B*), reportedly cause a dramatic decrease in BS[13] compared with that observed for UV-A*. However, relatively little natural UV-B in sunlight reaches the Earth's surface. Thus, UV-A appears to be more important for mechanically strengthening spider draglines, and for maintaining the mechanical functions necessary for capturing insects in orb webs during a fixed period of exposure to sunlight.

Noctural spiders

In contrast to the diurnal spiders, UV-A* irradiation affected the stress–strain behavior of draglines secreted by a nocturnal *Y. sia* spider weighing 153 mg (Figure 1b) by markedly decreasing both the BS and the BN values. Figures 2 and 3 show the time-dependent BS and BN changes of the *Y. sia* draglines after UV-A* irradiation, respectively. The BS and BN both decreased exponentially as the period of irradiation increased, suggesting that the orb webs of *Y. sia* had relatively weak mechanical resistance to UV-A* rays in terms of their mechanical breaking energy. This result suggests that UV-A* rays decrease the BS and BN of the orb webs of *Y. sia* spiders during the daytime, thereby weakening their mechanical function and making them unsuitable for capturing insects. Indeed, every evening these spiders emerge from their sheltered resting places, collect their previous orb webs and build new ones. The BS and BN of the orb webs of *Y. sia* spiders remain constant during the night because they are not exposed to UV irradiation. Thus, their mechanical properties do not decline overnight unless the orb webs are damaged by invaders.

UV-A* irradiation decreased the BS and BN values of the draglines of a nocturnal *N. nautica* spider weighing 203 mg, supporting the hypothesis that the draglines of nocturnal spiders are relatively weak against UV-A rays. However, the degree of decrease for the *N. nautica* spider was small compared with that of the *Y. sia* spider.

Dragline samples from both diurnal and nocturnal spiders, which were used in this study and kept under laboratory conditions without UV irradiation showed no changes in BS or BN over a period of

3 months. This constancy demonstrates that the absence of UV irradiation does not affect the BS and BN of the draglines.

Lifestyle change

Our findings imply that it is unnecessary for nocturnal spiders to secrete silks with strong resistance to UV rays. However, if such a species changes its lifestyle and adopts diurnal habits, they need to produce silk with sufficient UV resistance to preserve the mechanical function of the orb webs and allow them to trap insects. Though nocturnal *Y. sia* and *N. nautica* spiders are active at night, *Y. sia* spiders leave their orb webs and retreat to a sheltered position during the daytime, but *N. nautica* spiders are on their orb webs even in the daytime. This lifestyle difference may reflect the difference in the time dependence of the BS and BN after UV-A* irradiation. The silk of diurnal spiders could thus have developed their mechanical functional resistance to UV rays in association with a lifestyle change.

DISCUSSION

The experimental results indicate that diurnal *N. clavata, L. blanda* and *A. bruennichii* spiders use UV-A rays to mechanically strengthen the silks of their orb webs or to lengthen the period of time spent repairing and rebuilding their webs, whereas nocturnal *Y. sia* and *N. nautica* spiders do not employ these mechanisms.

The particular relationship between the change in the BS of silks of *N. clavata* spiders and the period of rebuilding their orb webs is discussed below. The time to reach a peak in the BS from the starting point showed a close correlation with the span, which is defined as the time during which the BS is higher than that at the starting point of UV irradiation. The span may give an indication as to the time period during which the orb webs should be rebuilt.

Even though UV rays were continuously irradiating the spider's draglines in the experiments presented here, UV rays actually irradiate the orb webs discontinuously due to the cycle from day to night and due to clear and cloudy weather. If sunlight in Japan is, on average, incident on the orb web for about 14 h in summer and about 11 h in autumn per day, the spans of 28 h for UV-A* rays may be estimated to be about 2 days. The estimated span corresponds roughly to the actually observed period of 2 days needed to rebuild the orb web by *N. clavata* spiders outdoors.[13]

UV-A rays contribute to producing crosslinks between protein molecules in spider silks for *N. clavata* spiders, and diurnal spiders effectively utilize UV-A rays to either mechanically strengthen the orb webs or lengthen the period between rebuilding to capture insects. This period is be affected by UV-A irradiation.

However, it is unnecessary for the silks of nocturnal *Y. sia* spiders to be strengthened by UV rays during the night. Indeed, the BS and BN of the silks of nocturnal *Y. sia* did not change under the absence of UV rays corresponding to the conditions at night, although the BS and BN decreased markedly with UV rays corresponding to the conditions in the daytime. This change suggests that nocturnal spiders never utilize UV rays for strengthening orb webs and also never use previous orb webs. Thus, nocturnal *Y. sia* spiders have to rebuild their orb webs every evening because UV rays destroy the orb webs in the daytime. In this study, three kinds of diurnal spiders were studied. However, nocturnal *N. nautical* spiders were neglected, except for *Y. sia*, because it was very difficult to accurately determine which spiders are nocturnal using only eyesight at night.

UV-A* irradiation induced the electrification of draglines even though no cracks in the surface were detected using scanning electron microscope photos. Actually, UV rays produce radicals[11,12,16] ascribed to the chemical cleavage of proteins. If UV-A* rays easily induce many cracks in the surface of draglines, it will not be easy for diurnal spiders to live safely during sunlit hours. The results suggest the chemical composition[12] that does not strongly alter the surface structure of draglines.

Another theory should be considered. The stronger BS of the silk of diurnal *N. clavata* spiders could be attributed to crosslinks between protein molecules.[10] This theory could account for the observed peaks of the BS as a consequence of the superposition of two factors: a decrease in the molecular weight caused by UV-induced chemical composition[17,18] and an increase in the molecular weight caused by UV-induced crosslinks.[19,20] Infrared and Raman spectra were obtained to ascertain the existence of crosslinks in the draglines after UV irradiation. However, no signals ascribed to crosslinks in the spectra were detected using these methods, and it is very difficult to detect crosslinks even with infrared and Raman spectra. Thus, it will be difficult to deny the theory that the difference in the mechanical strength between two kinds of spider silks may be ascribed to crosslinks. The difference in the mechanical behavior after UV irradiation may also be ascribed to the difference in the amino-acid composition or fine structure; or this difference may be ascribed to the hardening due to evaporation of water contained in spider draglines.[21]

In a previous paper,[12] electron resonance measurements revealed that photo-irradiation produced C_α-centered radicals of the protein molecules constituting the draglines, which was attributed to the breaking of chemical bonds. Greater numbers of radicals were induced by UV irradiation in mature spiders than in juveniles. The low levels of absorbance of UV radiation by the draglines of juveniles of *N. clavata* might be responsible for their stronger resistance to UV irradiation. The yellowing of draglines ascribed to the absorption at $\sim$275 nm may induce the production of radicals. In this study, however, the color of the draglines from nocturnal *Y. sia* and *N. nautical* spiders was white, and no absorption at wavelengths between 320 and 400 nm corresponding to UV-A* rays was observed. Thus, the peptide bonds ascribed to the amino acids constituting draglines secreted from nocturnal and diurnal spiders should be considered. It is possible that UV-A* rays markedly destroy the specific peptide bonds constituting draglines, although the peptide bonds were not examined here. The difference in the mechanical resistance to UV rays between diurnal and nocturnal spiders may be ascribed to the difference in the sequence of amino acids.

The results provide evidence for the hypothesis that diurnal spiders are at a more evolved stage than nocturnal spiders, because they produce silks that are mechanically strong against UV rays, even though the difference in the UV resistance is not yet clear in terms of the chemistry.

CONCLUSION

The findings imply that diurnal spiders cope with sunlight irradiation by effectively utilizing UV-A rays to mechanically strengthen their orb webs or by lengthening the period of time spent repairing and rebuilding their webs, whereas nocturnal spiders do not employ these mechanisms. The period of UV-A irradiation is important for these processes. In addition, if the quantity of UV-B rays reaching the ground is increasing due to the breakdown of the ozone layer and could have a significant effect on diurnal spider species in the future.

To conclude, this study provides strong evidence to support the hypothesis that *N. clavata* spiders have adapted to a diurnal lifestyle through mechanisms that are absent in nocturnal *Y. sia* spiders, such as the evolution of the strong mechanical functional resistance of their silk against UV rays, and further provides an important clue for obtaining silks with such mechanical functions from the evolutionary standpoint. Furthermore, knowledge of the evolution of mechanical functions provides an important way to search for biological materials with resistance to UV rays.

It is possible that UV-A* rays markedly destroy the specific peptide bonds constituting draglines, but the peptide bonds were not examined. The difference in the mechanical resistance to UV rays between diurnal and nocturnal spiders may be ascribed to the difference in the design for the sequence of amino acids. The process of chemical decomposition ascribed to UV-A* irradiation is now being studied in terms of the molecular weight.[22] In the near future, the hypothesis will be tested by examining the molecular distribution of the decomposed peptides prepared from spider draglines after UV irradiation.

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