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# A COMPARATIVE STUDY ON THE COLOR VISION OF FOUR COLEOPTERAN INSECTS

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**Jin-Tun Lin and Chin-Yih Wu** (1992) A comparative study on the color vision of four coleopteran insects. *Bull. Inst. Zool., Academia Sinica* **31**(2): 81-88. The electroretinograms (ERGs) and spectral sensitivities of the beetles *Coccinella septempunctata, Liocola brevitarsis, Cicindela specularis* and *Cicindela japonica* were investigated. The ERG responses from the compound eyes of all four coleopteran species were found to be negative and monophasic.

The ERG amplitudes of *C. septempunctata* and *L. brevitarsis* were larger than those of *C. specularis* and *C. japonica.* However, light intensity for 50% of maximal response obtained from *V*-log *I* curves in the former were lower than those of the latter for the same stimulus.

The spectral sensitivity curves of all four species showed two peaks: one near the UV band (360-380 nm) and the other in the green band (510-530 nm) of the spectrum. Because the two peaks match the Dartnall nomogram, it is suggested that at least two types of photoreceptors (UV and green) probably exist in the compound eyes of all four species. A third type of photoreceptor (between UV and green) existing in *C. septempunctata* was proven by selective monochromatic adaptation.

The relationship between the sensitivities of compound eyes to light and the different habitats of beetles, and the relationship between spectral types and body color patterns are discussed.

**Key words:** Color vision, Compound eyes, ERG, Spectral sensitivity, Coleoptera.

Ever since behavioral observations showed that bees are able to distinguish many different colors (Frisch, 1914), physiologists have been investigating the color vision of compound eyes in insects. Color vision depends on the spectral properties of photoreceptor types in compound eyes. At present, several methods have been used to study spectral sensitivity in insect compound eyes to determine receptor types. However, obtaining spectral sensitivity curves from ERG recordings is regarded as the easiest and most meaningful method (Agee *et al.*, 1990; Lall *et al.*, 1985; Wu, 1989).

Beetles (Coleoptera) are the most numerous in number and in species among insects. Their habits and habitats are so complicated that they have very different appearances, body colors, and eyes across species. However, there have been few electrophysiological accounts concerning the color vision of coleopteran compound eyes. For the present study, the spectral sensitivities of compound eyes in four species of beetles were investigated by using ERG to determine spectral types of photoreceptors. The relationship between the sensitivities of compound eyes to light and their habitats, and the relationship between spectral types and body color patterns will be compared and discussed in this paper.

## MATERIALS AND METHODS

Ladybird beetles, *Coccinella septempunctata* and tumble bugs, *Liocola brevitarsis* were collected in the field. The former can be maintained for months in the laboratory on a diet of aphids or drone honey bee powder, the latter for weeks on corn fruit. Tiger beetles, *Cicindela japonica* (Thunberg) and little tiger beetles, *Cicindela specularis* (Chaudoir) were collected in mountain regions and maintained for several months on a diet of small insects. The beetles were kept at 22°C and reared under an L:D 12:12 photoperiod.

For experimental preparation, insects were immobilized by cooling. Their legs and jaws were removed, their necks were sandwiched between two fine insect pines on a lucite case, and their heads and thoraxes were fixed to the lucite wall with bee's wax to prevent movement. A fine tungsten wire used as a recording electrode was attached to the central corneal surface of the right compound eye for ERG recording. Light emitted by a xenon arc lamp (150 watt) was passed through a monochromator and focused on the eye. The duration of the illumination was controlled by a shutter, with a flash duration of 300 ms and an interval between flashes of 10 sec to give a reasonable period for preventing light adaptation The energy of the monochromatic light was measured by radiometer. The reference intensity (log $I=0.0$) corresponded to $4.03\times10^{11}$ quantra/cm$^2$/s at all wavelengths ranging from 300 nm to 700 nm. The indifferent electrode was placed in the animals' thoraxes. EGR response signals were directly coupled to amplifiers and monitored by oscilloscope, then recorded on thermal recording paper.

For selective chromatic adaptation, two separate light paths were used. The adapting light was regulated by interference filters and focused on the eye for over one hour; the testing stimulus was then performed along with that used for dark adaptation measurements. Ommatidia counts of compound eyes were obtained from scanning electromicroscopic photographs.

## RESULTS

### Comparison of the ERGs of four coleopteran species

All ERG responses recorded on the central surfaces of right compound eyes of *C. septempunctata, L. brevitarsis, C. japonica* and *C. specularis* were monophasic negative waves (Fig. 1 inset). ERG response amplitudes depended on stimulus intensity, wavelength, and species of beetle. The potentials of the ERGs in response to green (530 nm) were usually larger than those to UV in all tested species under the same stimulus conditions. The amplitude of ERG potentials from *C. septempunctata* and *L. brevitarsis* were usually larger than those from *C. japonica* and *C. specularis* in both short and long wavelengths, even though *C. japonica* have many more ommatidia on each compound eye (Table 1).

### Comparison of visual sensitivity to white light

A 500 watt xenon arc lamp was used as a white light source; the intensity of the light stimulus was controlled using

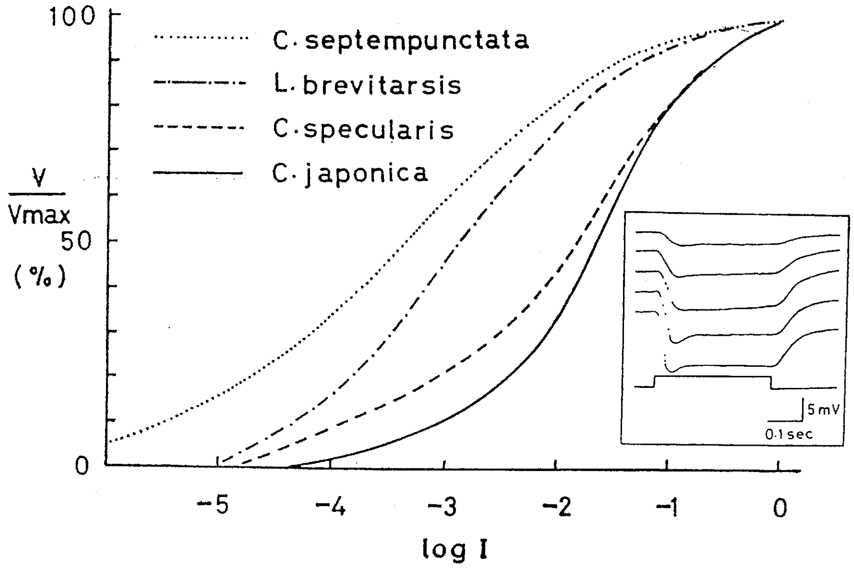


Fig. 1. A comparison of standardized stimulus response (*V*-log *I*) curves of four coleopteran species. The inset shows five ERGs recorded from *C. septempunctata* in response to light stimuli with 0, 1, 2, 3, and 4 log attenuating filters.

neutral density filters. ERG responses were recorded, and the *V*-log *I* curves (the relationships between response amplitudes and stimulus intensity from the compound eyes of the four species of beetle) were measured. All standardized *V*-log *I* curves for the four coleopteran species were sigmoidal in shape (Fig. 1). However, the *C. Septempunctata* and *L. brevitarsis* curves were separated by the *C. japonica* and *C. specularis* curves. The *K* values (intensities for 50% of maximal responses of *V*-log *I* curves (Eguchi and Horikoshi, 1984)), were very different between the two pairs about one log unit in intensity (Table 1, Fig. 1). In other words, the intensities causing the 50% of maximal responses to light for *C. japonica* and *C. specularis* were ten times more intense than those for *C. septempunctata* and *L. brevitarsis*.

**Comparison of spectral sensitivity curves**

Spectral sensitivity was measured from the spectral efficiencies obtained by stimulation with monochromatic flashes of the same quantal content. In the present experiment all spectral sensitivity

Table 1
A comparison of facets, ERG amplitudes and sensitivities to light in the compound eyes of four coleopteran species

| Insect | Facets/eye | ERG (mV) | | *K* values |
|---|---|---|---|---|
| | | 360 nm | 530 nm | (log *I*) |
| *C. septempunctata* | 980±50 | 10.85±0.60 | 12.26±0.80 | −3.38±0.12 |
| *L. brevitarsis* | 3,500±80 | 3.05±0.60 | 6.96±1.40 | −2.84±0.70 |
| *C. specularis* | 1,100±80 | 0.47±0.08 | 1.29±0.08 | −1.82±0.09* |
| *C. japonica* | 5,300±150 | 0.57±0.09 | 1.52±0.08 | −1.62±0.68* |

mean±SE ($N=6$); * $p<0.01$

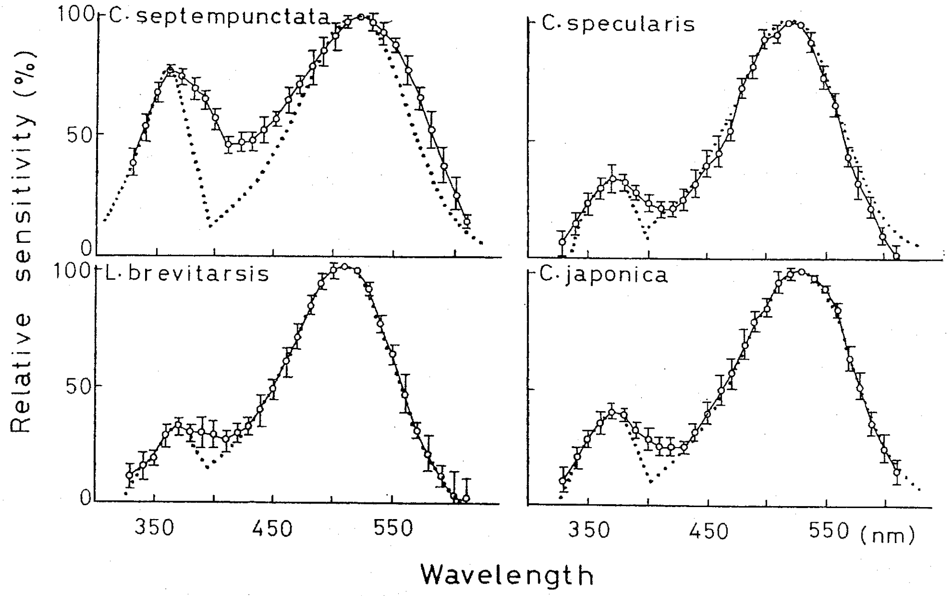


Fig. 2. A comparison of spectral sensitivity curves of dark adapted compound eyes. Dotted lines represent nomogram curves; vertical bars represent standard errors from eleven samples.

curves calculated from the *V*-log *I* curves of ERGs of the four coleopteran species had two peaks: one near the UV band (360–380 nm) and the other in the green band (510–530 nm) of the spectrum. All of the curves disappeared in the red band (above 600 nm) (Fig. 2). The relative sensitivities of all UV peaks were smaller than the green peaks. That the UV and green peaks match the nomogram curves (Dartnall, 1953) suggests that at least two kinds of photoreceptors (UV and green) exist in the compound eyes of all four species. When compared with the maximum peak, the relative sensitivities in the blue band (400–420 nm) of *C. japonica*, *C. specularis*, and *L. brevitarsis* were low, not above 20% of the maximum peak. However, the green and UV peaks of *C. septempunctata* were broader than the Dartnall nomogram (Fig. 2). Furthermore, the blue band in the curve appeared at about 50% of the maximum peak. It is reasonable to consider that a third type of photoreceptor between the UV and green peaks probably exists in the compound eyes of *C. septempunctata*. In order to prove this assumption, we performed the following experiments.

**A third type of photoreceptor exists in the eyes of *C. septempunctata***

We tested to see whether blue photoreceptors exist in the compound eyes of beetles by looking at selective chromatic light adaptation (selectively reducing the sensitivities of the ERG to one of the spectrum bands by light adapting the eyes with constant background illuminations of blue or green). We also measured the complete spectral sensitivities, adapted to both darkness and to backgrounds of 450 nm and 550 nm. After adaptation to selective chromatic light for one hour (to backgrounds of blue or green light), spectral sensitivities shifted downward along the ordinate axis about one log unit (Fig. 3A). The blue adapting light made the blue area and green peak less prominent than they were in the dark-adapted curve (Figs. 3A', 3B). On the other hand, the green adapting lights

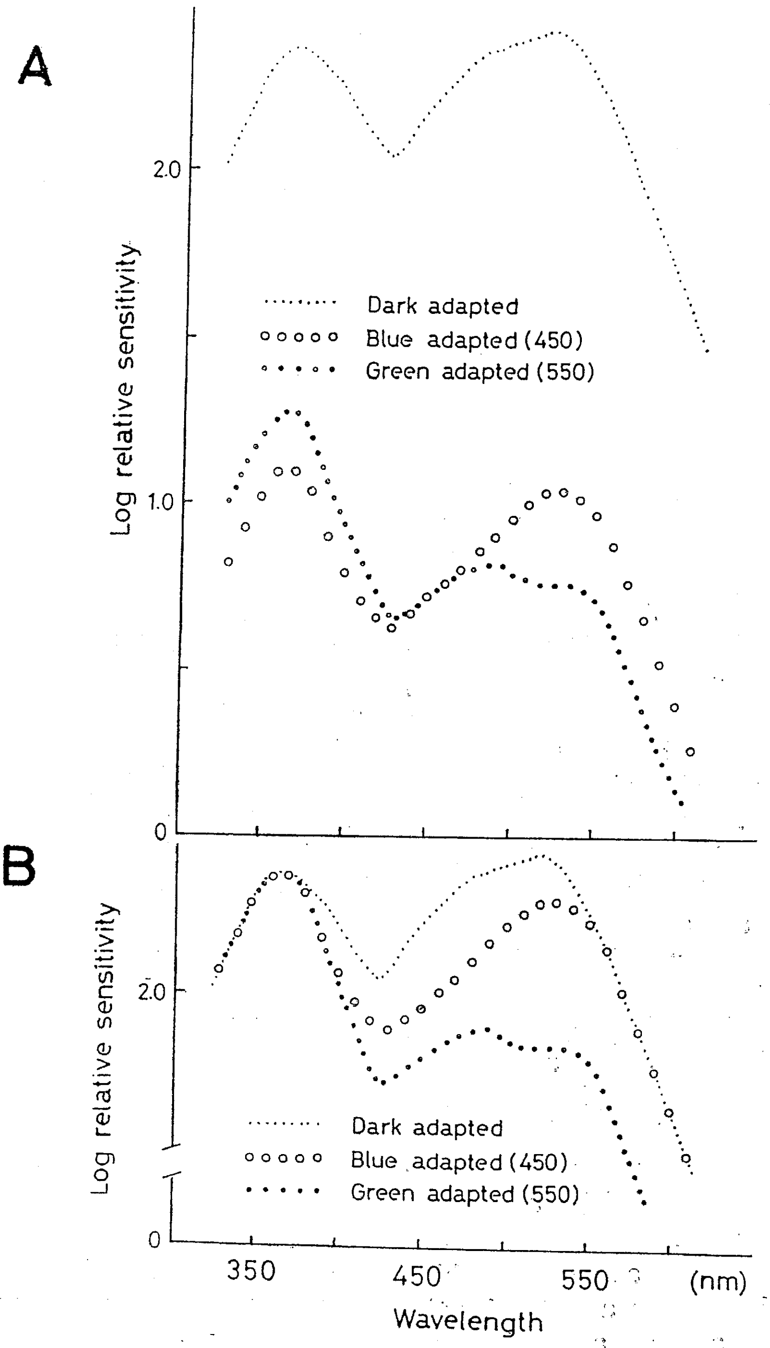


Fig. 3. Spectral sensitivity curves of the compound eyes of *C. septempunctata* in darkness (dotted line) and in 450 nm (open circle line) and 550 nm (closed circle line) adapted backgrounds. A: Sensitivity curves obtained from 450 nm and 550 nm adapted backgrounds shifted downward about one log unit after exposure for one hour. B: To compare changes of relative sensitivities affected by adaptation, the 450 nm and 550 nm adapted curves were artificially moved up and placed overlapping with the UV peak.

enhanced the blue area. That is, the green adapting lights reduced the green peak, but convexed the curve in the blue region of the spectrum (Fig. 3A, B). We then also tried to simultaneously test both 360 nm and 550 nm chromatic adaptation, but could not record results. These data suggest that the eyes of *C. septempunctata* may have a third type of photoreceptor near the blue region of the spectrum.

## DISCUSSION

The results of our experiments suggest that the compound eyes of all four coleopteran species consist of at least two different types of receptors: UV and green. That the compound eyes of *C. septempunctata* have a third type of photoreceptor was proven by selective chromatic light adaptation. Unfortunately, we could not get evidence on whether or not the third type of photoreceptor exists in the eyes of the other three species by the same method. They may have only two types of photoreceptors, the same as cockroaches (Butler, 1971), crickets (Lall *et al.*, 1985), and ants (Labhart, 1986); those three insects have been shown to have only two types of color receptors —UV and green —in their compound eyes. If the other three coleopteran species also have the third type of photoreceptor, the number of third type cells may be fewer than those of UV or green. Therefore, ERG responses—which are mass responses from all color receptor cell types within recording range—from third type cells may have been affected by the other type cells, and as a result were difficult to test by selective chromatic light adaptation. This problem will eventually be resolved using intracellular recording methods.

There are two types of ERG potential waves in the compound eyes of insects: one is slow, monophasic, and negative, and the other is rapid and biphasic (Autrum, 1958). The former has been found in butterflies and moths (Eguchi and Horikoshi, 1984), and the latter in oriental fruit flies (Wu, 1989). ERG response patterns of compound eyes in beetles belong to the monophasic and negative type, although the biphasic type was occasionally recorded due to electrode position. In the present study, the monophasic and negative ERG response amplitudes were used as data, since negative ERG components correspond to retinal receptor potentials (Autrum, 1958).

Several reports have demonstrated that several bright, colorful butterflies have a fourth color receptor with spectral sensitivity in the red region of the spectrum (Horridge *et al.*, 1983, 1984; Matic, 1983). Another colorful butterfly, *Papilio xuthus* has been reported to have a pentachromatic color vision system with spectral wavelengths of 360 nm (UV), 400 nm (violet), 460 nm (blue), 520 nm (green), and 600 nm (red) (Arikawa *et al.*, 1987). Those authors posited that the compound eyes of insects with bright, colorful appearances may have more kinds of color receptors than those with monochromatic appearances. *Cicindela japonica* have brighter color patterns on their bodies in comparison to *C. specularis.* The upper surface of elytra on the former is green and dark blue, interspersed with coppery red transverse bands and some mid-area white spots. The latter is gray with several small white spots on its elytra margins. However, these two species of beetle have the same set of receptor types (UV and green). On the other hand, *C. septempunctata* has a plainer color pattern (an orange-yellow back with seven black spots), yet it has three types of photoreceptors in each compound eye. Based on these results, the color vision system of colorful beetles may be very different from that of colorful butterflies. Hence, color receptors type in coleopteran visual systems is independent of body appearance.

Due to differences in habit and habitat, beetle eyes vary in size and shape. *C. japonica* have pairs of large, protruding compound eyes with several thousand ommaditia each; they are usually found on bare ground in mountain regions or dry riverbeds, and are abundant in sunshine. In summer they can be found on bare ground from about 9:00 a.m. until they disappear at around 6:00 p.m.; moreover, their time of greatest activity appears to be from 1:00 to 2:00 p.m. (Hori, 1982). On the other hand, *C. septempunctata*, which prey on aphids and mites that are found under plant leaves, are always found attached to plants and generally inactive in the shadows of leaves even in daytime. Their eyes are small and flat, with only a few hundred facets on each compound eye. Therefore, these habit and habitat differences may be related to their differences in ERG response amplitudes and visual sensitivity to light ($K$ values of $V$-log $I$ curves).

In conclusion, our results indicate that although these beetles have very different body color patterns they have identical sets of photoreceptors with maximum sensitivity to UV and grêen. However, differences in visual sensitivity to light are related to differences in habits and habitats.

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## REFERENCES

Agee, H. R., E. R. Mitchell and R. V. Flanders (1990) Spectral sensitivity of the compound eye of *Coccinella septempunctata* (Coleoptera: Coccinellidae). *Ann. Ent. Soc. Amer.* **83**: 817-819.

Arikawa, K., K. Inokuma and E. Eguchi (1987) Pentachromatic visual system in a butterfly. *Naturwis* **74**: 297-298.

Autrum, H. (1958) Electrophysiological analysis of the visual systems in insects. *Expl. Cell Res. Suppl.* **5**: 426-439.

Butler, R. (1971) The identification and mapping of spectral cell types in the retina of *Periplaneta americana*. *Z. vergl. Physiologie* **72**: 67-80.

Dartnall, H. J. A. (1953) The interpretation of spectral sensitivity curves. *Brit. Med. Bull.* **9**: 24-30.

Eguchi, E. and T. Horikoshi (1984) Comparison of stimulus-response ($V$-log $I$) functions in five types of lepidopteran compund eyes (46 species). *J. Comp. Physiol.* **154**: 3-12.

Frisch, K. von (1914) Der farbensinn und formensinn der biene. *Zool. J. Physiol.* **37**: 1-238.

Hori, M. (1982) The biology and population dynamics of the tiger beetle *Cicindela japonica* (Thunberg). *Physiol. Ecol. Japan* **19**: 77-212.

Horridge, G. A., L. Marcelja, R. Jahnke and T. Matic (1983) Single electrode studies on the retina of the butterfly *Papilio*. *J. Comp. Physiol.* **150**: 271-294.

Horridge, G. A., L. Marcelja and R. Jahnke (1984) Colour vision in butterflies. I. Single colour experiments. *J. Comp. Physiol.* **155**: 529-542.

Labhart, T. (1986) The electrophysiology of photoreceptors in different eye regions of the desert ant *Cataglyphis bicolor*. *J. Comp. Physiol.* **158**: 1-7.

Lall, A. B., E. T. Lord and C. O. Trouth (1985) Electrophysiology of the visual system in the cricket *Gryllus firmus* (Orthoptera, Gryllidae): Spectral sensitivity of the compound eyes. *J. Insect Physiol.* **31**(5): 353-357.

Matic, T. (1983) Electrical inhibition in the retina of the butterfly *Papilio*. I. Four spectral types of photoreceptors. *J. Comp. Physiol.* **152**: 169-182.

Wu, C. Y. (1989) Receptors in insects II. Electroretinogram of the compound eye in the oriental fruit fly (*Dacus dorsalis* Hendel). *Bull. Inst. Zool., Academia Sinica* **28**(1): 7-13.

# 比較四種甲蟲複眼的色光感覺

林金盾　　吳京一

以電生理學的方法，研究甲蟲複眼的網膜電圖 (Electroretinogram, ERG) 及分光感度 (spectral sensitivity)，發現七星瓢蟲 *Coccinella septempunctata* 和白星花潛金龜子 *Liocola brevitarsis* 的複眼網膜電圖，均比小斑蝥 *Cicindela specularis* 和斑蝥 *Cicindela japonica* 爲大，而且對光刺激之反應敏感度也較高。四種甲蟲複眼的分光感度曲線均具有兩個尖峰：一個在短波長區 (360–380 nm)，另一個在長波長區 (510–530 nm)，而且各尖峰均與 Dartnall 標準色素曲線相吻合，得知複眼中至少含有感受紫外光和綠色光等兩種色覺視細胞。使用選擇適應法驗證結果，證得七星瓢蟲的複眼，除紫外光和綠色光外，還可能含有第三種感受藍色光 (400–420 nm) 的色覺視細胞。本文對甲蟲的光刺激反應敏感度與其生活習性和棲息環境的關係；甲蟲體表鮮艷程度與色覺視細胞種類的關係，加以討論。