

## AN ECOLOGICAL CORRELATE OF CRITICAL FLICKER-FUSION FREQUENCIES FOR SOME *ANOLIS* LIZARDS

THOMAS A. JENSSEN and BETTY SWENSON<sup>1</sup>

Biology Department, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061, U.S.A.

(Received 15 October 1973; in revised form 12 November 1973)

**Abstract**—The maximum critical flicker-fusion frequency (CFF) was determined for seven species of anoline lizards. The congeners showed a wide range of CFF values from 26 to 42. These values correlated with the general level of insolation in each species' microhabitat, such that the brighter the habitat, the higher the CFF.

### INTRODUCTION

The flicker-fusion phenomenon has been known for some time and has been extensively investigated using human Ss (Henkes and van der Tweel, 1964; Landis, 1953). However, CFF determinations for most vertebrate species are unavailable. For lizards, such data are few (Crozier and Wolf, 1939, 1941). More importantly, the ecological and evolutionary significance of CFF have been largely neglected.

This paper presents the maximum CFF for seven species of *Anolis* lizards. These data have utilitarian value when using the motion picture technique as an experimental tool in behavioral research (Jenssen, 1970). However, of more theoretical interest is that the results of this comparative study provide impetus to consider the adaptive nature of the flicker-fusion phenomenon as an evolved characteristic in response to a species' ethoecology. The ecological adaptiveness of CFF is reflected in the wide range of maximum values found for different species within the same genus.

### METHODS AND MATERIALS

To determine the CFF, a classical optokinetic response was utilized. When placed in a rotating environment, a lizard will move its head or eyes in the direction of rotation, followed by a rapid counter-rotational movement. To elicit this head and eye nystagmus, lizards were placed inside a rotating, transparent cylinder with vertical black stripes on it. To create flashes of light, a translucent curved screen was placed outside the revolving cylinder which also had vertical black stripes of the same width and spacing as the cylinder. As the drum was slowly accelerated or decelerated, a particular rotational speed was reached which produced cessation or initiation of head and eye nystagmus; this point of behavioral transition provided a threshold index to whether

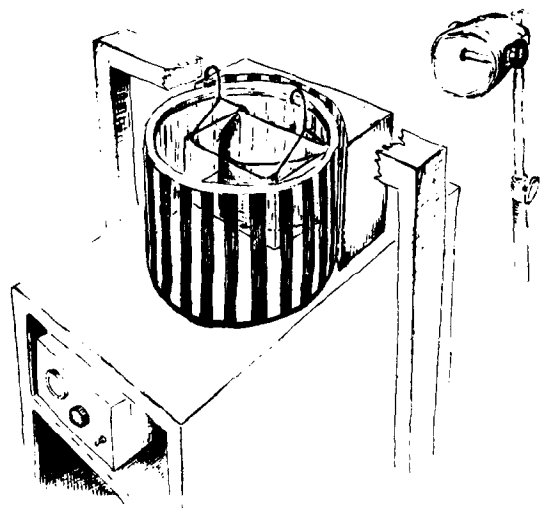


Fig. 1. The optokinetic apparatus used for estimating CFF values (heat filter and cover to holding box not shown).

or not the lizard was perceiving a rotating environment. The number of flashes/sec being delivered at the moment of this behavioral transition determined the CFF value.

The experimental apparatus was composed of several components (Fig. 1). The lizard was placed in a 30.5 × 12.3 × 12.3 cm clear plastic box. The bottom and top of the box were opaque except for a small viewing port in the cover. A semi-cylinder of white pasteboard inside the box blocked all lateral angles of view except through the front wall. The holding box was suspended from an overhead support. On the underside of the support member was fitted a mirror that allowed the investigator to monitor the lizard's eye and head movements through the overhead viewing port. The holding box support structure straddled a platform that held a motor-driven disc. Upon the latter was bolted a drum with sides constructed of 0.2-mm-thick transparent celluloid sheeting. The drum was 40 cm (dia) × 31 cm (ht) and had black vertical stripes 2 cm in width and placed 2 cm apart around its circumference.

<sup>1</sup> Present address: Department of Biology, College of William and Mary, Williamsburg, Virginia 23185.

Table 1. Means and S.E. of the means for critical flicker-fusion frequency determinations of some *Anolis* lizards using two methods

Species tested	Drum speed during CFF determinations	
	Decreasing	Increasing
<i>A. lineatopus neckeri</i>	26.1 ± 0.51	25.6 ± 0.70
<i>A. limifrons</i>	28.3 ± 0.19	27.9 ± 0.24
<i>A. valencienni</i>	33.4 ± 0.66	35.1 ± 0.65
<i>A. carolinensis</i>	34.5 ± 0.42	33.8 ± 0.34
<i>A. grahami</i>	34.6 ± 0.34	33.5 ± 0.36
<i>A. sagrei</i>	34.4 ± 0.28	34.5 ± 0.24
<i>A. auratus</i>	42.0 ± 0.73	37.8 ± 0.83

Screwed to the platform next to the drum was a striped background screen which was of the same materials, pattern, and height as the drum. The screen curved in a 180° semi-cylinder around the drum, and was positioned about 2 mm from the drum. The front viewing wall of the holding box suspended within the drum faced the background screen. A quartz-King 500 W floodlight, fitted with a heat filter, was positioned 0.66 m away from the holding box with its beam directed through the stationary striped screen, the rotating drum, and onto the front window of the holding box. A red filter across the screen served to diffuse and decrease the illumination, and to provide long wavelength radiation for maximal stimulation of the subject's cone receptors (see below).

The drum was turned by a motor-driven belt. Control of rotation speed was maintained through a rheostat. The drum speed was registered by a tachometer. The tachometer, however, was only a convenience as its accuracy was not considered consistent enough. The actual number of rotations/time unit that produced *S*'s CFF value were counted directly.

The CFF determination was begun by placing *S* in the dark for 5 min within the suspended holding box. A longer period of dark adaptation was not deemed necessary considering the high light intensity used in the experiment and the fact that, for humans at least, dark adaptation has been found to have a small (Brown, 1965) to no effect (White and Baker, 1971) upon CFF for a given light intensity. The drum was then spun well above that speed which would produce the experimental animal's flicker fusion threshold. The floodlight was turned on and the lizard was watched in the mirror as the drum gradually slowed. As soon as the lizard began nystagmic movements the floodlight was switched off and the number of seconds required for the drum to make 20 rev was recorded. The number of seconds divided by 20 rev produced drum rev/sec. The rev/sec value was multiplied by 32 (32 pairs of opaque and transparent stripes on the drum) to yield flashes/sec at the initiation of nystagmus, thus providing an estimate of a lizard's CFF. Five runs were made with each animal.

The above procedure was also run by starting the drum from a standstill and accelerating gradually until nystagmic movements ceased. With the exception of *A. valencienni*, this method produced similar, but generally slightly lower, CFF values (Table 1). The lower values may be due in part to habituation (Hayes, Hertzler and Hoberg, 1968). For the purposes of this paper, the data obtained by decreasing the drum speed will be stressed.

A variable that affects CFF is the wavelength of the deli-

vered light. Rods and cones of the retina have different CFF characteristics and respond differently to given wavelengths of light. The photopic receptors (cones) are more sensitive to long wavelength radiation than to short, and have the highest CFF values of the rod-cone receptor systems (Pieron, 1965). Since anoline lizards possess cone bearing retinas (Polyak, 1957; Tansley, 1957; Underwood, 1970; Walls, 1942), red light was predominantly used to obtain the highest expected CFF values. A qualitative and quantitative analysis was made of the light which entered the holding box via an ISCO Model SR spectroradiometer fitted with a remote probe, and is shown in Fig. 2.

A lizard's CFF is also dependent upon light intensity. With increasing illumination, the CFF value increases and follows a sigmoid curve, being asymptotic at an upper limit of light intensity. In our experiments, all CFF determinations were made at 218  $\mu\text{W}/\text{cm}^2$  (Fig. 2) at a luminosity intensity of 353 lx; this latter value was derived from the data in Fig. 2 using weighting factors to relate spectral energy to luminosity for the various wavelengths. The light intensity employed in the present study is estimated as being greater than that used in the experiments of Crozier and Wolf (1939, 1941) when they determined the maximum CFF for the lizards *Sphaerodactylus inaguae* and *Phrynosoma cornutum*. Presumably our methods produced maximum CFF values for all tested species.

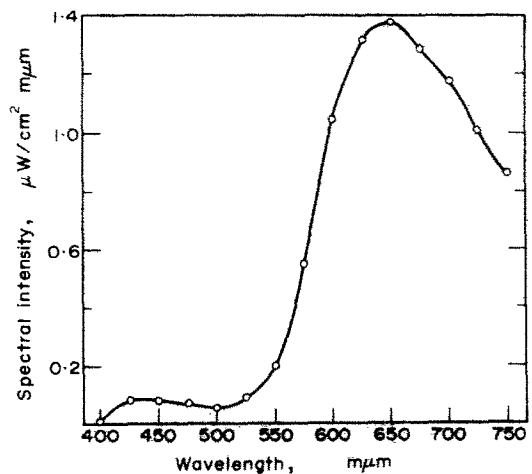


Fig. 2. Quantitative and qualitative analysis of light delivered to experimental animals for CFF determinations.

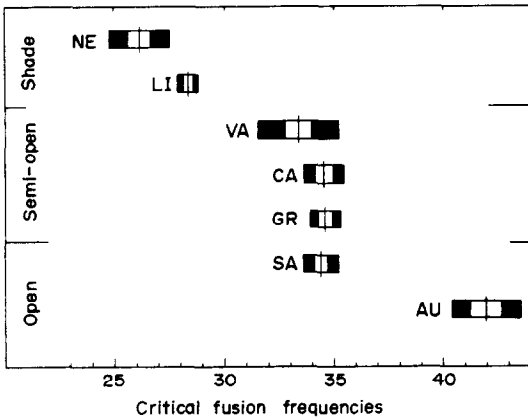


Fig. 3. Mean CFF values determined for *Anolis* lizards, *lineatopus neckeri* (NE), *limifrons* (LI), *valencienni* (VA), *carolinensis* (CA), *grahami* (GR), *sagrei* (SA), and *auratus* (AU). Sample statistics are as follows: vertical line is the mean, outer edges of the adjacent white boxes denote the standard error of the mean, and outer edges of the black boxes delimit the 95 per cent confidence limits of the mean.

The experimental animals were all adult male lizards of the genus *Anolis*. This genus contains well over 200 species; its members demonstrate considerable ecological radiation. Below is outlined for each studied species its taxonomic position within the genus (Etheridge, 1960) and a few generalizations about its distribution and habitat characteristics.

*A. auratus*—three specimens; collected in Panama; belongs to the beta group, *chrysolepis* species series; species distributed in Central America and northern South America; lives in grassy areas of direct sun exposure (*open, grass*).

*A. sagrei*—two specimens; collected in Jamaica; belongs to the beta group, *sagrei* species series; species distributed in Cuba, Bahamas, Jamaica, southern Florida, eastern Mexico, and Central America; lives in open habitats of fence posts, low bush, and low tree trunks (*open, ground-trunk*).

*A. grahami*—six specimens; collected in Jamaica; belongs to the beta group, *grahami* species series; species distributed in Jamaica, Cayman Islands, and Bermuda; lives in semi-open habitat from fence posts to upper tree trunks (*semi-open, trunk-crown*).

*A. carolinensis*—five specimens; collected in Florida; belongs to the alpha group, *carolinensis* species series; species distributed in southeastern United States and Bahamas; lives in semi-open habitats from shrubs to arboreal perch sites (*semi-open, bush-trunk-crown*).

*A. valencienni*—one specimen; collected in Jamaica; belongs to the beta group, possibly the *sagrei* species series; species distributed in Jamaica; lives in semi-open habitat near or on trees from low trunk to small diameter perches in the crown (*semi-open, crown*).

*A. limifrons*—five specimens; collected in Panama; belongs to the beta group, *fuscoauratus* species series; species distributed in Central America and northern South America; lives in shade on low understory vegetation of closed canopy forests (*shade, bush*).

*A. lineatopus neckeri*—one specimen; collected in Jamaica; belongs to the beta group, *grahami* species series; species distributed in Jamaica; lives in heavily shaded areas from shrubs to low on tree trunks (*shade, bush*).

Table 2. Sorted table of mean maximum CFF values from a SNK test run on five repeated measurements on each of 23 individuals representing seven species of *Anolis* lizards

Species tested	Mean CFF	Non-significant ranges between means
<i>A. l. neckeri</i>	26.1	-----
<i>A. limifrons</i>	27.9	
<i>A. limifrons</i>	28.1	-----
<i>A. limifrons</i>	28.2	
<i>A. limifrons</i>	28.4	-----
<i>A. limifrons</i>	29.1	
<i>A. carolinensis</i>	33.0	-----
<i>A. valencienni</i>	33.4	
<i>A. grahami</i>	33.4	-----
<i>A. sagrei</i>	33.8	
<i>A. carolinensis</i>	33.9	-----
<i>A. grahami</i>	34.1	
<i>A. grahami</i>	34.2	-----
<i>A. grahami</i>	34.6	
<i>A. carolinensis</i>	34.8	-----
<i>A. carolinensis</i>	34.8	
<i>A. sagrei</i>	35.0	-----
<i>A. grahami</i>	35.6	
<i>A. grahami</i>	35.9	-----
<i>A. carolinensis</i>	36.1	
<i>A. auratus</i>	39.4	-----
<i>A. auratus</i>	43.0	
<i>A. auratus</i>	43.5	

## RESULTS

The CFF values were determined for 23 anoline lizards, representing seven species, and are shown in Fig. 3. A single classification analysis of variance comparing CFF values within and among individuals was statistically significant ( $df = 22/92$ ,  $F = 24.21$ ,  $P < 0.001$ ) and attributed 82 per cent of the observed variance to comparisons between lizards and 18 per cent to within individual comparisons. These data were also tested for significant deviations from the assumptions pertaining to normal distributions. With the exception of data collected from one *A. carolinensis*, distribution of the recorded values showed no significant platy- or leptokurtotic trends. Goodness-of-fit and homogeneity of variance tests were also run and the resulting adjusted  $\chi^2$  and  $F_{\max}$  values indicated that the sample observations were normally distributed. The data met the above criteria for administering parametric tests.

After the data were analyzed, the *a posteriori* SNK test (Sokal and Rolf, 1969) was run which showed three distinct groupings (Table 2). The first of these three groups was of the shade-dwelling *A. l. neckeri* and *A. limifrons* individuals whose species mean CFF values were 26.1 and 28.4, respectively. Though the CFF values for these two species were statistically different (Fig. 3), the CFF values from individuals of these samples were close enough in magnitude for the SNK test to show some overlap (Table 2). The mean CFF of *A. valencienni* (33.4), *A. carolinensis* (34.5), *A. grahami* (34.6), and *A. sagrei* (34.4) made up the second group that was discriminated by the SNK test. With the exception of *A. sagrei*, these are all of the tested species which live in microhabitats of intermediate-intensity insolation (semi-open habitat). *Anolis sagrei* was the only species to deviate from the hypothesis that the maximum CFF value reflects the intensity of sunlight upon a species' microhabitat. The third group established by the SNK test consisted of *A. auratus*, a species living in open, grassy areas, having a mean CFF of 42.0.

## DISCUSSION

Considering their close systematic relationship, the mean critical flicker-fusion frequency (CFF) for seven species of anoline lizards showed a surprising range of values (26.1–42.0). These values did not correlate when comparing the species with their species series or their geographic distributions. However, the relative intensity of microhabitat insolation did correlate with the mean CFF values. With the exception of *A. sagrei*, there was a consistent trend for a species' CFF value to be higher if the anole lived in a microhabitat of greater exposure to direct sunlight. This correlation, however, may even apply to *A. sagrei*. This species is a successful colonizer of Cuban origin (Williams, 1969), with presumably broad ecological tolerances. During

the relatively recent colonization of Jamaica, *A. sagrei* has invaded the open, ground to low elevation structural habitat in which there are no sympatric competitors. Yet on Cuba, the *A. sagrei* populations are significantly more arboreal than on Jamaica (Williams, 1969). Hence, the intermediate CFF value of the Jamaican *A. sagrei* very likely reflects the arboreal habitat of intermediate light intensity in which the ancestral stock on Cuba may have evolved.

From the above interspecific correlation of CFF and habitat illumination, it appears there are differing selection pressures responsible for shaping the flicker-fusion phenomenon in anoline lizards. The CFF value of a species' visual system is presumably adaptive. At the present time, however, the reasons for this correlation remain speculative. Nevertheless, a hypothesis is developed below.

From an ecological viewpoint, the CFF reflects the proficiency to resolve moving objects. The faster an animal's CFF, the shorter is the persistence time of the afterimage as an image moves across the retina. A high CFF value means that perception of a moving object is less likely to be blurred, and determination of its nature, direction, and velocity is facilitated (Walls, 1942). An animal's CFF, then, would be related to its ability to quickly negotiate a complex habitat, identify and catch swift prey, and avoid fast-moving predators. Some of these suggestions are posed by Protasov (1970). In his comparative study of fish species, Protasov found that mullet, black sea anchovy, and atherinid had high CFF values (55, 63, 67, respectively). These were the species studied which fed on moving organisms and were potential prey of fast-moving predators (i.e. dolphins and bonitos).

However, these lines of speculation are not directly applicable to the present set of saurian congeners. With the possible exception of *A. valencienni*, all tested anoles have the same general characteristics regarding locomotion, prey acquisition, and predator avoidance. Moreover, the speed of the prey and predators encountered by the tested species is not sufficiently different to support this hypothesis.

Having a high CFF would seem universally advantageous. However, in explaining the wide spread of *Anolis maximum* CFF values, the usual sacrifice of visual acuity to gain sensitivity in animals evolved for low light intensities may be a likely causal agent. Rods characteristically have lower maximum CFF values than cones (Krill, 1964; Pieron, 1965). In their study of CFF determinations for the horned lizard, *Phrynosoma cornutum* (pure cone retina), and the gecko, *Sphaerodactylus inaguae* (pure rod retina), Crozier and Wolf (1941) found *P. cornutum* has a CFF value (42) 48 per cent faster than *S. inaguae* (26) under bright illumination (100 mL). However, an additional finding of significance was that the *S. inaguae* CFF drops slowly in magnitude with decreasing illumination, while the *P. cornutum* CFF value decreases rapidly. In dim light (2 mL), *S. inaguae* actually has a greater CFF than *P. cornutum*. Presumably as light intensity decreases, the

gecko is able to carry out its social, feeding, and predator avoidance needs more efficiently than the horned lizard because the gecko can maintain a visual acuity for moving objects which becomes superior to that of the diurnal horned lizard in dim light.

Such a situation as outlined in the above example may also exist for *Anolis*. The shade-dwelling *A. l. neckeri* has a maximum CFF almost the same as *S. inaguae* (26.1 and 26.8, respectively), and the maximum CFF for *A. auratus* and *P. cornutum* are also very similar, being 42.0 and 42.4, respectively. Furthermore, from field observations made by the senior author in Jamaica, *A. l. neckeri* remains active into the crepuscular period, with territorial males even giving social displays with their dewlaps (throat fans). These activities were taking place at light levels barely sufficient for human vision. Color vision by *O* was not possible, but the movement of the *A. l. neckeri* light lemon colored dewlap was evident. The other syntopic congeners had retired almost an hour before ambient illumination fell to this level.

What is needed now is an understanding of how the CFF of shade-adapted and sun-exposed anoles respond to low light levels. However, if a difference in functional response to low light intensities is found among anoles similar to that described for *S. inaguae* and *P. cornutum*, interpretation may be difficult. Unlike the comparison of the gecko with the horned lizard, all the *Anolis* studied so far have been found to possess solely cone-bearing retinas (Underwood, 1970). However, Underwood (1970) has described three different types of cones from anoline retinas; possibly one of these receptor types may be functioning in a rod-like manner. It would be interesting to see if the proportion of these three cone types significantly varies when making comparisons of shade and sun-dwelling anoline species.

*Acknowledgements*—Roger L. Kaesler (University of Kansas) recommended and made available the statistical procedures followed in this paper. Gordon Burghardt (University of Tennessee), Jack Hailman (University of Wisconsin), and A. Stanley Rand and Michael Robinson (Smithsonian Tropical Research Institute) contributed valuable criticism of the manuscript. John Cairns, Ed Hover, and Orson Miller (Virginia Polytechnic Institute and State University) kindly loaned some of the equipment and provided assistance for the study.

To these people we are most grateful for their time and aid. We are also appreciative of the financial assistance from the National Geographic Society for helping make this investigation possible.

## REFERENCES

- Brown J. L. (1965) Flicker and intermittent stimulation. In *Vision and Visual Perception* (Edited by Graham C. H.), pp. 251–320. Wiley, New York.
- Crozier W. J. and Wolf E. (1939) The flicker response contour for the gecko (rod retina). *J. Gen. Physiol.* **22**, 555–566.
- Crozier W. J. and Wolf E. (1941) The flicker response contour for *Phrynosoma* (horned lizard; cone retina). *J. Gen. Physiol.* **24**, 317–324.
- Etheridge R. E. (1960) The relationships of the anoles (Reptilia: Sauria: Iguanidae): An interpretation based on skeleton morphology. Univ. of Michigan Ph.D. Thesis.
- Hayes W. N., Hertzler D. R. and Hogberg D. K. (1968) Visual responsiveness and habituation in the turtle. *J. Comp. Physiol. Psych.* **65**, 331–335.
- Henkes H. E. and van der Tweel L. H. (1964) Flicker. *Proc. Symp. physiology of Flicker and Proc. of the 2nd Symp. of the Int. Soc. for Clin. Electroretinogr.* W. Junk, The Hague.
- Jensen T. A. (1970) Female response to filmed displays of *Anolis nebulosus* (Sauria, Iguanidae). *Anim. Behav.* **18**, 640–647.
- Krill A. E. (1964) A technique for evaluating photopic and scotopic flicker function with one light intensity. In *Flicker* (Edited by Henkes H. E. and van der Tweel L. H.), pp. 452–461. W. Junk, The Hague.
- Landis C. (1953) *An Annotated Bibliography of Flicker-Fusion Phenomenon Covering the Period 1740–1952*. Univ. of Michigan Press, Ann Arbor, Michigan.
- Pieron H. (1965) Vision in intermittent light: laws and mechanisms of the critical frequency for fusion. In *Contributions to Sensory Physiology* (Edited by Neff W. D.), pp. 179–264. Academic Press, New York.
- Polyak S. L. (1957) *The Vertebrate Visual System*. Univ. of Chicago Press, Chicago.
- Protasov V. R. (1970) *Vision and Near Orientation of Fish*. Israel Program for Scientific Translations Ltd., Keter Press, Jerusalem.
- Sokal R. R. and Rohlf F. J. (1969) *Biometry: the Principles and Practice of Statistics in Biological Research*. W. H. Freeman, San Francisco.
- Tansley K. (1957) *Vision in Vertebrates*. Chapman & Hall, London.
- Underwood G. (1970) The eye. In *Biology of the Reptilia* (Edited by Gans C.), pp. 1–97. Academic Press, New York.
- Walls G. L. (1942) *The Vertebrate Eye and its Adaptive Radiation*. Cranbrook Institute of Science, Bloomfield Hills, Michigan.
- White K. D. and Baker H. D. (1971) Changes in the foveal CFF during the course of dark adaptation. *Paper presented at Psychonomic Society*, St. Louis, October, 1971.
- Williams E. E. (1969) The ecology of colonization as seen in the zoogeography of anoline lizards on small islands. *Quart. Rev. Biol.* **44**, 345–389.

*Résumé*—On détermine la fréquence critique maximum de fusion (CFF) sur sept espèces de lézards *Anolis*. Les congénères présentent un grand domaine de valeurs de CFF entre 26 et 42. Ces valeurs sont en corrélation avec le niveau général d'insolation pour le microhabitat de chaque espèce, la CFF croissant avec la luminance de l'habitat.

**Zusammenfassung**—Das Maximum der kritischen Flimmerverschmelzungsfrequenz (CFF) wurde für sieben Eidechsenarten bestimmt. Die Werte von Tieren gleicher Art zeigten CFF-Werte zwischen 26 und 42. Diese Werte waren mit dem allgemeinen Niveau der Sonneneinstrahlung am jeweiligen Standort jeder Art korreliert, d.h. je höher der Standort war, desto höher waren die CFF-Werte.

**Резюме**—Максимум критической частоты слития световых мельканий (КЧМ) был определен для семи видов ящериц.

Было показано, что КЧМ у этих видов изменяется в широких пределах от 26 до 42. Эти величины коррелировали с общим уровнем инсоляции микросреды обитания данного вида; таким образом, чем выше яркости в месте обитания, тем выше КЧМ.