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FEMALE RESPONSE TO FILMED DISPLAYS OF *ANOLIS NEBULOSUS* (SAURIA, IGUANIDAE)

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Lizards of the family Iguanidae possess stereotypic behaviours which many times incorporate sexually dimorphic colour patterns and structures. In *Anolis* the large size and spectacular colouration of the dewlap is characteristic of the males of most species (Etheridge 1959). The dewlap functions as an integral part of the anoline display which undoubtedly is used as a means of communication. However, the type of information conveyed by these visual displays and their social significance have long been subjects of speculation.

Investigations of the function of lizard displays in mate selection have been restricted for the most part to non-experimental approaches. In his book, *The Origin of Species*, Darwin reasoned that the development of sexual dimorphism in males was caused by females choosing to mate with those males having the most prominent ornamentation. Following this hypothesis, Mertens (1926) suggested that the large and brilliantly coloured dewlaps of male anoles serve to attract females to their own species. This idea had a large following. However, from their observations of lizard courtship and mating, Noble & Bradley (1933, p. 86) suggested that the bright colours of the male anoline dewlaps evolved as frightening devices, and do not stimulate the female to mate. Evans (1938a, b) disagreed with the latter interpretation, believing the male display of *Anolis sagrei* and *A. carolinensis* is very important in sexually stimulating the female.

Actual attempts to test experimentally the effect of the male's display-action-pattern (DAP) upon the female have been few. Only the investigations of Greenberg & Noble (1944), Harris (1964), and Hunsaker (1962) have contributed to this area of study. Much more objective data must be gathered before a realistic appraisal can be made of the role, if any, of the male's display in species recognition and mate selection.

In the present study a film loop technique was implemented which has advantages over methods used by previous investigators. Female *A. nebulosus* were presented with two colour films duplicated from the same filmed sequence of a displaying male. One of the film duplicates, however, was altered so that it no longer portrayed the DAP characteristic of *A. nebulosus*. The choices made by the female anoles to the projected displays provided some insight into the display's social significance as will be discussed. The film loop technique described here for the first time has wide application for the behaviourist as a tool for future experimental studies.

Methods

From the behavioural repertoire of *A. nebulosus*, the 'assertion' display seemed to have the greatest potential to serve as a species recognition display, and thus possibly function during mate selection. Of the four basic display patterns employing the dewlap, the 'assertion' display is the most frequently seen in the field (Jenssen 1970). It is given by the adult males as they move about their territories, and when courting females. This display is also the most stereotyped of the four display patterns.

Before evaluating the function of the 'assertion' display, it was necessary to establish the dynamics of this pattern. From a thorough display analysis of a population of *A. nebulosus* (Jenssen 1971), a reliable estimate was obtained for the variability in this population's display. Such an estimate was mandatory if the basic display was to be altered for experimental purposes; otherwise there would be no point of reference for comparing the changed display with the normal condition.

Five male and thirty-one female *A. nebulosus* used in the following experiments were collected from the same population 35 km east of Tepic, Nayarit, Mexico, during the spring of 1967 and 1968. The sexes were held separately in 1·2 × 1·2 × 0·9 m enclosures. During most of the year the males had to be kept in the dark or else many would have been lost due to territorial fighting.

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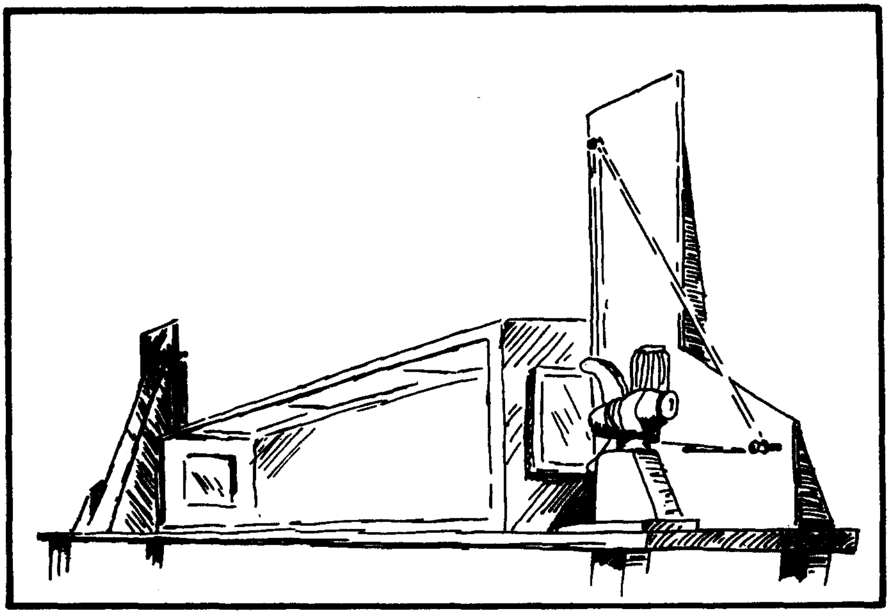

Fig. 1. Film loop apparatus.

Experimental Apparatus

A frame was designed to hold a 16-mm cinema projector and three flanged idler wheels which supported a film loop (Fig. 1). Two of these film-loop frames were constructed of 1-cm thick plywood. They were placed at both ends of a 1·8 × 0·6 × 0·6-m cage. The latter was constructed of an angle iron frame with a glass panel composing the front wall, sliding screen wire doors forming the top, and the remaining sides of the observation chamber made of masonite (Fig. 1). Rear projection screens were fitted to the ends of the chamber where the film-loop frames were positioned. The observation cage contained a sand-filled floor, a centrally placed release box and water dish, and oak leaves with a few branches at both ends by the projection screens. A 1·2 × 1·2 × 1·5-m blind was also made. To ensure absolute concealment of the observer, the blind was light proofed except for several short, 1-cm wide observation slots. A flashlight furnished the light needed for note taking. Within the blind were switches to control the running of the projectors.

Two Bell and Howell 16-mm Analyst projectors (Model 173) were used with the film loops. This model projector allowed considerable flexibility. The projection speed was variable from approximately six to over twenty-four frames per second by means of a calibrated rheostat. Still, forward, and reverse projection were possible. An iris in one of the projector's lenses permitted the illumination of the projected displays to be matched so that no bias resulted from one image being brighter than the other. The size of the projected images were also matched by adjusting the distance of the projector from the screen. Sponge rubber pads were placed beneath the film loop apparatus to deaden vibrations. Suppression of projector noise, however, was not attempted. If this noise in any way influenced the lizard's behaviour, its effect was not detected.

The film sequence which was used as the standard stimulus in all film-loop experiments was taken with Kodachrome II indoor film at twenty-four frames per second. This sequence was of a large male performing an 'assertion' display from a simple perch. During the filming the lighting was set so that only the subject was illuminated and the background remained unexposed. Thus, when the resulting film was projected upon a dark screen, a 'framing' effect was eliminated and only the lizard and his perch were seen. A few branches were placed under the projected image, producing an exceedingly realistic effect. In addition, the screen itself was recessed 4 cm into the enclosure's inside wall to enhance the three-dimensional illusion.

A number of duplicates were made from the original filmed display. From most of these copies, alterations of the original display were produced. These altered displays were created by adding and/or removing certain parts of the display. The resulting film strips were again duplicated to eliminate the splices. Each film loop was constructed by splicing together the ends of a filmed sequence and threading it onto the film loop apparatus.

Methods Evaluation

Some theoretical considerations should be given to the feasibility of using motion pictures in behaviour studies. The illusion of motion obtained from a rapid progression of separate pictures results from the visual system fusing these photos into a continuum. The particular point at which the eye no longer sees individual pictures or flashes is known as the critical fusion frequency (CFF). This is a characteristic phenomenon of the visual system which has been known for some time (Landis 1953).

Although much data concerning the flicker fusion phenomenon have been gathered (Henkes & van der Tweel 1964), there are very few reports of its manifestation in lizards. Crozier & Wolf (1939, 1941) determined the CFF for the gecko, *Sphaerodactylus inagual* (rod retina) and for the horned toad, *Phrynosoma cornutum* (cone retina); their critical fusion frequencies were 27 and 56 Hz, respectively. Conceivably, the CFF for *Anolis* is near the 56 Hz threshold found for the horned toad since members of both genera have many retinal features in common (Walls 1942).

The number of Hz produced by the proection apparatus is of utmost importance. The Bell and Howell projector used in the present investigation has a partial disc which rotates in front of the film gate aperture. The partial disc is geared so that each frame shown by the projector is interrupted or flashed three times. Thus, a film running at twenty-four frames per second, as was the case in these experiments, is flashed onto the screen at 72 Hz. This frequency is greater than the CFF for *P. cornutum* and presumably greater than the CFF of *A. nebulosus*. It is probable that the experimental animals of the present study were perceiving a moving image.

Another important consideration of anoline vision deals with colour. Not only do their retinas contain large numbers of cones (Walls 1942), but Tansley (1957, p. 97) reported that *A. carolinensis* can discriminate hues. Therefore, colour film was used in attempting to create a realistic stimulus.

However, a major problem encountered in utilizing a cinematic stimulus is the lack of a three dimensional image. This is particularly relevant to anoles. Underwood (1951) found the eyes of *Anolis lineatopus*, *A. grahami*, and *A. opalinus* possess two foveae, a feature shared only with some birds. The largest fovea is located centrally in the retina and is the area of fine vision during monocular operation; this is the primary mode of sight. The more shallow temporal foveae are used for binocular vision. A similar situation is described for *A. carolinensis* (Polyak 1957). Therefore, it is very likely *A. nebulosus* can also perceive depth. To what extent this limitation decreased the effectiveness of the experimental technique cannot be evaluated; however, behavioural observations did show the animals responded to the image as if it were real.

To evaluate the effectiveness of the film-loop technique, five preliminary tests were made using male *A. nebulosus*. The territorial male is a good subject because a predictable agonistic behaviour pattern is evoked in him by the presence of a displaying male. This behavioural indicator was the criterion for the film loop's success.

In the preliminary tests a male was placed in the observation chamber where after a short time he selected a particular habitat at one end of the chamber. After a 24-hr period, the normal display was projected onto the screen across the enclosure from the 'resident' male. Almost immediately he responded in a manner characteristic of aggressive encounters. The nuchal crest rose, his body became laterally compressed, the gular area was inflated slightly, and the light and dark areas of his body pattern became more contrasted. The male oriented himself laterally to the displaying image across the cage and began displaying. All five males involved in these tests crossed the cage, displaying on the way, and reached the illusionary lizard. Two males actually leaped at the 'challenging male' on the screen.

The advantages of the film loop, many of which are yet to be exploited, are the control of: (1) the sequential order of the units within the behaviour pattern by means of splicing techniques, (2) the image size, (3) the speed of the behaviour, (4) the initiation and repetition of the behaviour, and (5) the general colour of the image via use of black and white film and coloured filters. In addition, one has an exact replication of the animal and its behaviour which is much easier to produce than building an animated model of the species.

Experimental Procedure

Twenty female lizards were used in each of the experiments with the exception of the last. Before the trial, each female was held in the release box for 15 min to help eliminate any behavioural effects induced by handling. The door to the box was then slid open. Upon emergence the film loops were shown and the experimental animal was allowed 45 min to reach the habitat at either one end of the enclosure or the other; such a move constituted a choice, otherwise the trial was scored as no choice.

During each trial, two film loops were employed. Every run was made with the normal display as well as with an altered film loop. The projectors were alternately run with two display sequences being shown first from one projector and then two displays from the other. This procedure of continuously alternating projectors was adopted during initial testing; it was found the females spent more time observing the screened images when they appeared singly than when both displays appeared together.

From preliminary tests, it was also found that the addition of a clump of moss just in front of the release box was necessary. Without the moss, females emerging from the box onto the sand substrate spent little or no time examining their surroundings, but rather bolted for the oak leaves distributed at the ends of the enclosure. This reaction is in accord with field conditions, where females show strong habitat preference for leaf litter and are rarely seen in open areas (Jenssen 1970). With the moss present, the female lizards remained for several minutes in this semi-protective cover at the centre of the cage. This behaviour allowed them time to observe both displays.

Two experimental designs were established for the four experiments. The first experimental design was used with experiment 1, while experiments 2, 3, and 4 followed the second design.

Experiment 1. Each of twenty females was given three consecutive trials with a 15 min rest period between runs. Two normal displays were presented except one was shown in reverse sequence (Fig. 2C). After each trial the projectors' polarities were switched so that the backward display never appeared on the same screen twice in succession.

Experiments 2, 3 and 4. In these experiments each female ran only one trial per experiment. During each experiment the altered display was projected onto one screen for the first half of the trials and then switched to the opposite screen for the remaining females. The film loops used in these experiments were as follows: experiment 2, normal display (Fig. 2A) and display containing no head nods and six dewlap pulses (Fig. 2D); experiment 3, both loops were of normal display, and experiment 4, normal display and display with only one dewlap pulse eliminated (Fig. 2B).

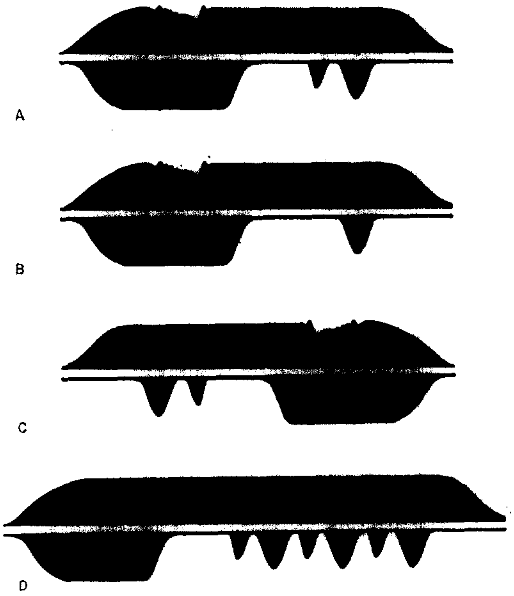

Fig. 2. Display patterns used in film loop experiments with female *Anolis nebulosus*. Upper block represents amplitude (vertical axis) and duration (horizontal axis) of head movement and lower block represents dewlap movement. A, normal display; B, C and D, altered displays.

Results

Female *A. nebulosus* were shown two filmed sequences of a displaying male of their own species. With the exception of experiment 3, one of the two projected displays was an alteration of the normal behaviour pattern. The females were allowed to choose between the displaying images (Table I).

In experiment 1 the altered display was a normal film loop run backwards. On the initial trial fourteen females chose the habitat of the

Table I. Number of Female *Anolis nebulosus* Making Moves in Response to Normal and Altered Film Loop Displays

Experiment	Female responses		
No. 1	Normal display	No choice	Altered display
Trial 1	14*	1	5
Trial 2	11	2	7
Trial 3	12	2	6
All trials	37*	5	18
No. 2	Normal display	No choice	Altered display
	14*	2	4
No. 3	Left side	No choice	Right side
	8	1	11
No. 4	Normal display	No choice	Altered display
	12	0	6

*Significantly greater frequency than that expected by chance.

normally displaying male while only five females entered the habitat of the image performing the reversed display. This 2·8 : 1 ratio (females toward normal display : females toward altered display) was statistically significant at the 5 per cent level as analysed both by the chi-square method and from the expected frequencies of a binomial distribution.

When the reversed display was switched to the opposite end of the enclosure for the second trial, a much more even split was observed (1·6 : 1). The ratio increased to 2 : 1 in the third trial when the reversed film loop was shifted back to its initial end of the enclosure. A 2·1 : 1 choice ratio resulting from the combination of all trials was statistically significant (5 per cent level).

The departure from the overall trend in the choice ratio of trial 2 cannot be unequivocally accounted for. There is evidence, however, which indicates that the deviation resulted from a secondary motivation of the female lizards. *A. nebulosus* females are very territorial and remain within relatively small areas under natural conditions (Jenssen 1970). During preliminary tests in which the film loops were not used, females exhibited a type of 'territoriality' and 'homing' within the observation cage. When released repeatedly within a short time span, the individual females would usually return to the end of the enclosure of their initial choice. If undisturbed, they would also remain in the chosen habitat of the chamber for at least 3 days (the longest period tested). Relevant to this discussion are the observations of Greenberg & Noble (1944, p. 431). They found that once a female *A. carolinensis* was residing in a male's territory, she was usually not induced to desert by an adjacent rival's display, even when the resident male's dewlap was held retracted by collodion. The experimental design of experiment 1 permitted this territorial tendency to show itself, and thus introduced a second variable. To eliminate this variable, a female was used for only one observation in each of the remaining experiments.

As in experiment 1, the altered film loop of experiment 2 bore little resemblance to the normal display. All of the head nods were removed and four dewlap pulses were added to the display sequence. The resulting choice ratio (3·5 : 1) was heavily weighted in favour of the normal display and was statistically significant (Table I).

Experiment 3 employed two identical normal display loops. This experiment was set up as a control to see if there was any intrinsic feature of the enclosure which in itself would attract the lizards. Eight females entered the left habitat and eleven chose the right side, which is close to a 1 : 1 ratio. No preference for one end of the enclosure was apparent.

The last experiment was particularly significant because the altered film loop was similar to actual display anomalies which occurred in approximately 5 per cent of the sampled population. One of the two dewlap pulses was eliminated from the filmed display sequence. Although the results were not statistically significant, the resulting choice ratio of 2 : 1 (probability of 0·12) possibly reflects some discrimination on the part of the females.

The female lizards used in this study were not all in the same stage of oestrus during the experiments, and at times some were obviously gravid (two or three per trial, mean of 2·3); these latter individuals accounted for most of the lizards in the 'no choice' category. They exhibited apparent rejection behaviour during exposure to the filmed male displays and were most unpredictable in their habitat choices.

Table II. Choice Ratios of Female *Anolis nebulosus* Computed from Data in Table I (Number of Females Entering Habitat of Standard Display—Number of Females Entering Habitat of Altered Display)

Experiment no.	Standard display	Altered display	Choice ratio
			Standard : Altered
3	Normal	Normal display	1·4 : 1
4	Normal	One dewlap pulse eliminated	2·0 : 1
1 (trial 1)	Normal	Normal display reversed	2·8 : 1*
2	Normal	Head nods eliminated six dewlap pulses	3·5 : 1*

*Statistical significance.

In general, the females gave a graded response to the film loops. When expressed in terms of choice ratios, there was an increasing proportion of animals attracted to the normal display as the altered display contained a greater number of changes from the generalized pattern (Table II).

Discussion

Several experimental studies have been made on species recognition and courtship behaviour in iguanid lizards (Ferguson 1966, 1969a, b; Pyburn 1955). However, very few investigators worked directly with the lizard display and its significance. Harris (1964) constructed a rough model of an *Agama agama* made out of two jointed wood blocks. By pulling a string, Harris could make the simulated lizard nod. When his model was painted the same colours and in the same pattern as a territorial male, it would elicit strong aggressive reactions from nearby dominant rainbow lizards. Gorman (1968), on the other hand, constructed a wooden model of *Anolis trinitatis* with appropriate body and dewlap colouration, but he drew no reactions from the anoles with his dummy lizard.

Greenberg & Noble (1944) investigated the effect of the colour of the male's dewlap upon the attraction of females. Working with *A. carolinensis* under semi-natural conditions, they allowed two males to establish territories on either side of a glasshouse. Branches connected both territories with a centrally situated release box. The release box had a glass top enabling the female inside to observe the displaying males. One of the males either had his pink dewlap coloured green, or had the extension of the dewlap prevented by a coat of collodion. After 3 to 5 min the box was opened and the female's choice recorded.

From the results of fifty-six trials, Greenberg & Noble (1944) concluded that the colour of the dewlap is not innately attractive to females. They believed, however, that the colour brings the attention of the females to the displaying male, and the display itself causes receptive females to gravitate toward the male.

Greenberg & Noble (1944, pp. 430–431) pointed out some difficulties with their procedure There were differences in the type and intensity of the male's behaviour as well as their proximity and visibility to the female. Lack of control over the males resulted in an inability to standardize the experiments completely.

Hunsaker (1962) also manipulated the male's display to gain insight into its function. He studied the *Sceloporus torquatus* group which lacks the large dewlap found in anoles; sceloporine lizards primarily perform push-ups and head-nods during their displays. Hunsaker attempted to show how the display-action-patterns act as an interspecific isolating mechanism. He presented the DAP graphs of seven species of *Sceloporus*, labelling them as species-specific. Some of these patterns were incorporated into a bobbing apparatus which consisted of a model lizard activated by a thread tied to a rod riding over the notches of a rotating wheel. A motor rotated the notched wheel at about 100 rev/min. The display data of *S. mucronatus* and *S. torquatus* were programmed onto separate wheels via the notches and used in the following experiments.

A 0·6 × 0·3 m enclosure was partially divided into three sections with a bobbing machine at both ends. Females were placed into the enclosure and random observations made to note in what chamber a female lizard was located. One bobbing machine was programmed with a display simulating that of the species of the female in the cage. The other bobbing machine contained a 'random' notched wheel, or one which simulated the display of another species which was represented in the cage by a second female. Five different experiments were run with a total of seven lizards.

Hunsaker observed the females most frequently in the chambers occupied by the model lizard which was simulating the females' species-specific bob. When the bobbing machines were shut off, he found the females distributed themselves randomly among the chambers. From these observations Hunsaker concluded that the species-specific display of the males served as a species recognition signal and permitted the females to establish territories close to males of their own kind.

The experimental methods used by Hunsaker could have been strengthened. His conclusions were based on the performance of only seven lizards. Each experiment consisted of many observations on not more than two animals. The bobbing machine used in the experiments had a motor which turned the notched display wheel at about 100 rev/min, causing the wheel to revolve completely once every 0·6 s. Yet the display-action-patterns of *S. mucronatus* and *S. torquatus* used for the display wheels were presented as being greater in duration than 0·6 s. Under such conditions there would be little or no pause between the displays, and it is improbable the experimental animals could discern where one display finished and the other began. Species recognition from the model lizard would seem difficult.

The papers cited above, as well as the present study, suggest that the male display of some lizards can attract the females of their respective species. Presumably, the display could serve as an ethological isolating mechanism in areas of cohabitation with closely related species (yet to be studied). It should be stressed, however, that these reports are only preliminary investigations, and that much more work must be done before more positive statements can be made. In his discussion of the role of visual communication in reproductive isolation, Marler (1961, pp. 96–97) mentioned how small the body of direct evidence is for a field where great advances are likely to be derived through an experimental approach. Perhaps the relative lack of experimental data can be attributed to the difficulty of assessing the real functions of ethological factors. Too frequently, unproven but often repeated hypotheses and casual observations have been substituted for needed research.

Summary

A film loop apparatus was devised to test a possible social function of the display of *Anolis nebulosus*. Films of a displaying male were projected onto translucent screens at both ends of an enclosure. One film loop portrayed the display typical of the lizard's population, while the other loop projected an altered version of the basic display. Female *A. nebulosus* were introduced singly into the centre of the enclosure. They gave a graded response to the film loops; an increasing proportion of females approached the 'normal' displaying image as the film loops of altered displays contained a greater number of changes. The various experimental techniques previously used to study the function of lizard displays are reviewed.

Acknowledgments

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