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Variation in Anal Spot Expression of Gambusiin Females and Its Effect on Male Courtship

ALEX E. PEDEN

Many species of the poeciliid genus *Gambusia* have a conspicuous concentration of melanophores near the female urogenital opening. This pigment or anal spot varies little in its expression in some species, but in others such as *G. affinis, G. georgei,* and *G. heterochir,* it varies considerably and is largest and most conspicuous at that time when eggs are present in the ovaries. When young ready for birth occur in females, anal spots are inconspicuous or absent. White artificial models of females were constructed. Dark anal spots were painted on these models and males were allowed to court them. Gonopodial imprints from courting males were recorded on a soft substrate on the model. Data from these experiments and observations indicate anal spots attract males and facilitate orientation when the gonopodium is thrusted at the female's urogenital opening.

FEMALES of many species of the poeciliid genus *Gambusia* have an anal spot (dark pigment near urogenital opening) which provides an excellent taxonomic feature to characterize species (Hubbs and Springer, 1957; Minckley, 1963). Because most fishes are cryptically countershaded with a dark dorsal surface and a pale ventral surface (Norman and Greenwood, 1963), such conspicuous pigment on a contrasting white ventral surface probably has a selective advantage that counteracts the disadvantage of increased visability of this pigment pattern towards predators. Since male *Gambusia* transfer sperm to the female's urogenital opening through the use of their modified anal fin or gonopodium (Rosen and Gordon, 1953), the hypothesis was tested that anal spots attract males to females and provide targets toward which males can direct gonopodia during copulation.

METHODS

To indicate variation in anal spots with the ovarian cycle, I coded pigment patterns of female *Gambusia* over 25 mm SL in a method similar to that employed by Hubbs (1959). The intensities vary from *1* for little or no pigment to *5* for a large, dark anal spot (Fig. 1). Ovary contents were recorded as: 1) "eggs," if the ova had no evidence of eye pigmentation; 2) "embryos," if eye pigment was present but the tail had not grown long enough to wrap around the circumference of the yolk to meet the head; or 3) "young," if the tail was longer and would meet the head when it was wrapped around the circumference of the yolk. If two or more stages of development were present, only the most advanced embryos were coded.

Live females anesthetized with M-aminobenzoic acid ethyl methanesulfonate salt (Sandoz Corporation) were observed under a microscope. Courting preference of males for live females with and without anal spots in stock aquaria were also recorded.

Preliminary observation revealed that male *Gambusia* would court artificial models. Molds for these models were constructed by embedding preserved females in silicone rubber aquarium sealer and then slitting one end of the mold to withdraw the fish when the sealer had hardened. The flexible rubber sealer permitted Plaster of Paris to be

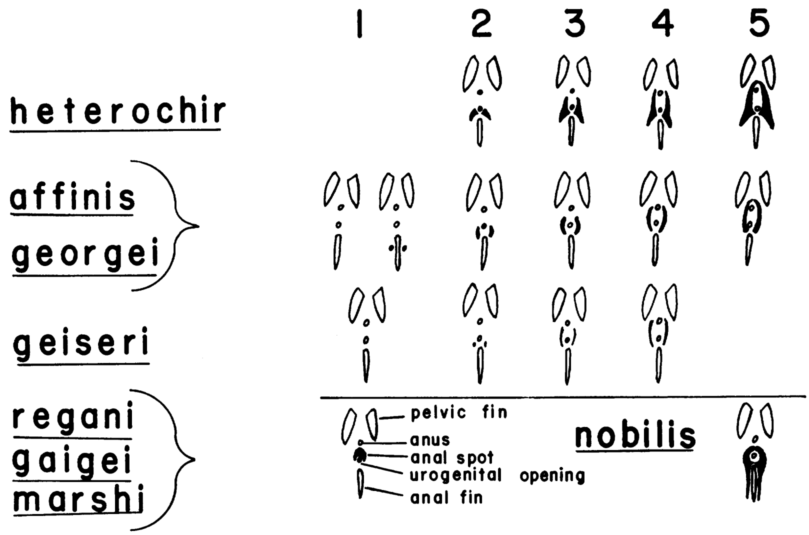

Fig. 1. Code for expressing different intensities of the anal spot in *G. heterochir, G. affinis, G. georgei,* and *G. geiseri* (see Figs. 7, 8, 9 and 10); and patterns of anal spots for *G. nobilis* and species of *Gambusia* which possess a median anal spot.

readily placed in the mold and, after setting, the newly-formed model was easily removed. An important feature was the creation of a hole of about 3 × 4 mm in the genital region (Fig. 2) so that cooking shortening could be placed in this hole. The soft shortening was used to record imprints of gonopodia when males thrusted toward the models. To prevent the Plaster of Paris model from dissolving when it was in the water, it was painted with a nonwater soluble lacquer (*i.e.,* clear fingernail polish). Anal spots were painted on the shortening with black india ink, but, because the ink clings poorly to shortening, the surface was first roughened to allow the ink to stick better. Several patterns of anal spots such as those shown in Fig. 3 were painted on the anal region of the model and presented to males. The letters under each pattern in Fig. 3 refer to the anal spot type.

After the ink had dried, the model was suspended in the aquarium by a white thread and male *Gambusia* were permitted to court it. In most tests the model was either drawn gently through the water past a male or the ventral area of the model was positioned near his snout, depending on which method attracted males better.

All models were produced from the same mold of a 42 mm SL female *G. affinis*; however, several models had to be used since they deteriorated rapidly and had to be replaced frequently. The model was modified in some instances. In a few experiments a piece of clear plastic cut in the shape of an anal fin was glued to the model (Fig. 2). The gill region was also hollowed out in some experiments and the hole filled with shortening so that an anal spot could be

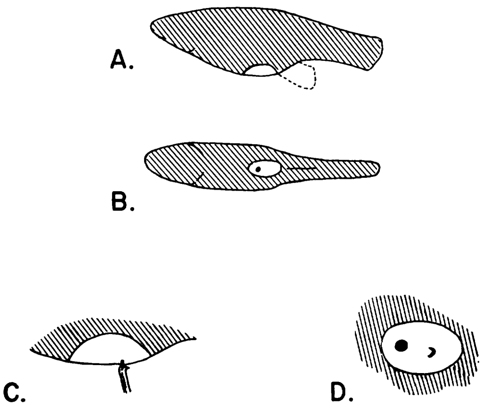

Fig. 2. Diagrams of artificial model: A, mid-sagittal section (dotted line indicates position of artificial clear plastic anal fin placed on some models); B, ventral view; C, mid-sagittal section through genital region of model (a gonopodium which is poking into the hollowed-out section that has been filled with shortening is shown); D, ventral view of genital region (the solid circle indicates an artificial "anal spot" applied to the shortening, the U-shaped mark is a gonopodial imprint in the shortening.

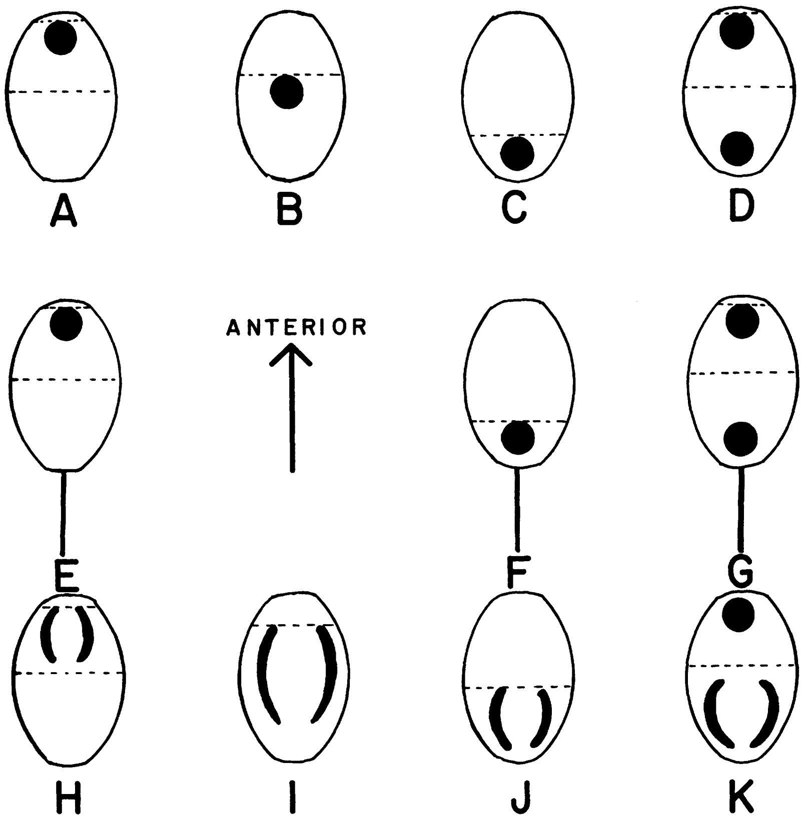

Fig. 3. Anal spot patterns on models which were presented to courting male *Gambusia*. Arrow points anteriorly. Horizontal dotted line divides the surface of shortening into zones on which gonopodial thrusts were recorded for each spot type (see Tables 1 to 4). In the middle row, the posteriorly directed lines indicate patterns in which an artificial anal fin was applied to the model (see Fig. 2-A).

placed at either an anterior or a posterior position of the same model (Fig. 4).

Males living with females or males isolated individually in aquaria thrusted their gonopodia at models relatively infrequently. Therefore to increase the motivation of males to court artificial models, several males which had had previous courtship experience were placed together in an aquarium and isolated from females for a week or more before the models were presented.

Because males usually thrusted at a model a few times and then ignored it in any one observation period, they were tested several times over several days to obtain data. In addition, males frequently thrusted but missed the shortening and thus left no gonopodial imprints so that the data recorded were only a fraction of the total number of gonopodial thrusts.

The gonopodial terminology and taxonomic nomenclature of Rosen and Bailey (1963) were used here except that *G. gaigei* and *G. hurtadoi* are given separate species status. *G. amistadensis* Peden (1973), *G. aurata* Miller and Minckley (1970), *G. georgei* Hubbs and Peden (1969), and *G. krumholzi* Minckley (1963) were named since Rosen and Bailey's publication.

Preserved and live specimens of *G. affinis* were obtained from San Felipe Creek, Val Verde Co.; Colorado River at Zilker Park in Austin, Travis Co.; Bull Creek, Travis Co.; Clear Creek, 17 km west of Menard, Menard Co.; and Llano River at Junction, Kimble Co., Texas. Preserved specimens and live stocks of *G. heterochir* from Clear Creek; *G. georgei* and *G. geiseri* from San Marcos, Hays Co.; *G. amistadensis* from Goodenough Spring, Val Verde Co.; *G. gaigei* from Big

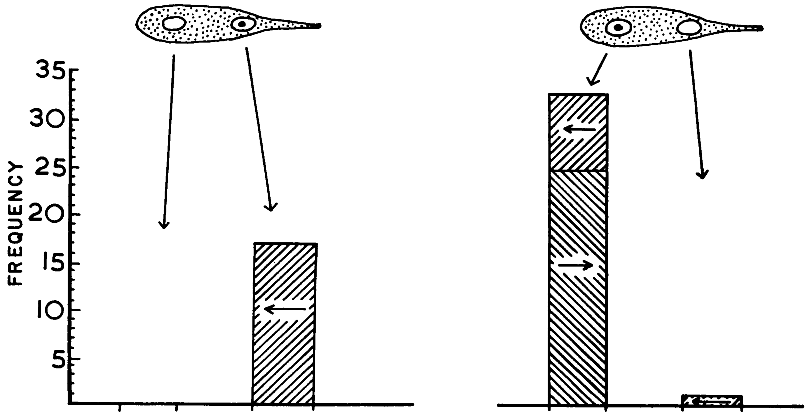

Fig. 4. Frequency of gonopodial imprints by male *G. aurata* on shortening at anterior and posterior end of model when an anal spot was placed either at the region of the gill isthmus or the normal genital area. The arrows within each bar indicate the direction in which the male approached the model.

Bend National Park, Brewster Co.; and *G. nobilis* from Phantom Lake and San Solomon Spring, Reeves Co., Texas, were also available for study. From Mexico, live and preserved specimens were used of *G. atrora* from Axtla, San Luis Potosi; *G. aurata* from Rio Guayaleja, Tamaulipas; *G. hurtadoi* from El Ojo de la Hacienda Dolores, Chihuahua; *G. krumholzi* from near Nava, Coahuila; *G. marshi* from Cuatro Cienegas, Coahuila; *G. regani* from near Padilla, Tamaulipas, and from Axtla, San Luis Potosi; *G. senilis* from the Rio Conchos at Saucilio and from between Jiminez and Hildalgo del Parral, Chihuahua; and *G. vittata* from Axtla and the Rio Guayaleja. Preserved collections of *G. panuco* from Rio Antiquo Morelas and Arroyo Terronoites at Highway 85 in southern Tamaulipas, Mexico, as well as *G. nicararguensis* from Honduras were examined. Live specimens of *G. caymanesis* from the Cayman Islands were used for comparison.

In order to minimize the inclusion of hy-

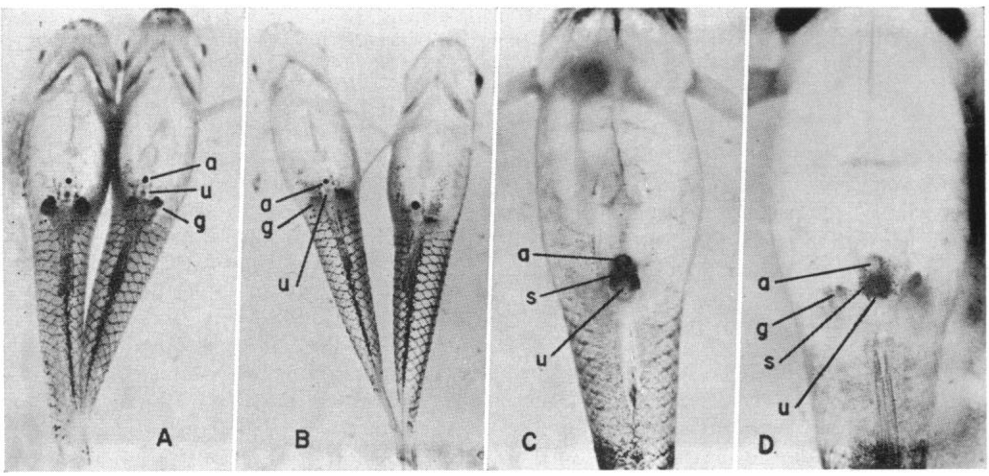

Fig. 5. Photographs of pigmentation in the genital region of *Gambusia* females: A, anesthetized *G. geiseri* females; B, same females as in "A" but photographed after being preserved in 10% formalin for 48 hours after their first photograph was taken; C, *G. gaigei* female recently preserved in 10% formalin; D, anesthetized *G. caymanensis* female. A = anus, u = urogenital sinus, g = gravid spot, and s = anal spot.

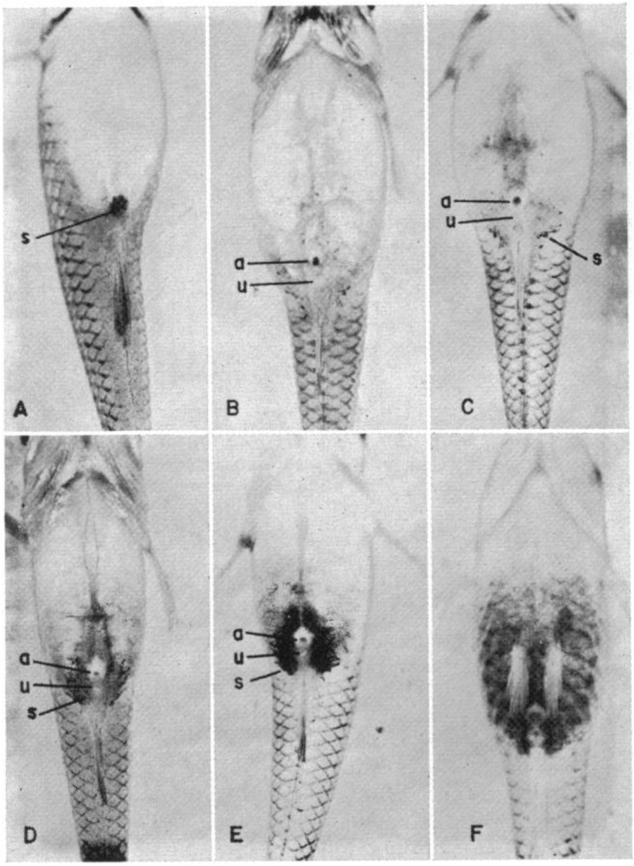

Fig. 6. Photographs of pigmentation in genital region of *Gambusia* females: A, anesthetized *G. krumholzi* female; B, anesthetized *G. heterochir* female which contains large embryos; C, same female as in "B" but photographed after preservation in 10% formalin for 48 hours after its first photograph was taken; D, anesthetized *G. heterochir* female which contains eggs only; E, same female as "D" but photographed after preservation in 10% formalin for 48 hours after its first picture was taken; F, preserved *G. heterochir* which contains eggs and has an unusually large anal spot. a = anus, u = urogenital sinus, and s = anal spot.

brids in the data on preserved specimens from Clear Creek, only *G. affinis* with either 6 dorsal or 13 pectoral fin rays and *G. heterochir* with either 8 dorsal or 15 pectoral fin rays were used.

Description of Anal Pigmentation

In general, pigment on the dorsal surface of the body of *Gambusia* originates from chromatophores in the outer integument, but the pigment on the ventral surface of the abdomen is caused mostly by the white "outer" surface of the peritoneum which lines the inner body cavity. Except for anal spots, the abdominal musculature and integument external to this peritoneum are translucent or transparent. Because the white peritoneum is interrupted by the passages of the urogenital and intestinal tracts from the body cavity to the exterior and the associated tissues of these ducts of living individuals are translucent or transparent, dark pigment is often visible through these openings. In addition, the peritoneum of some species often splits laterally, producing a similar source of pigment in the form of a gravid spot (Fig. 5). Such dark internal pigment originates from the melanin lining the "inner" surface of the peritoneum that surrounds the coelom and reproductive tract. The amount of visible dark pigment varies depending on the degree

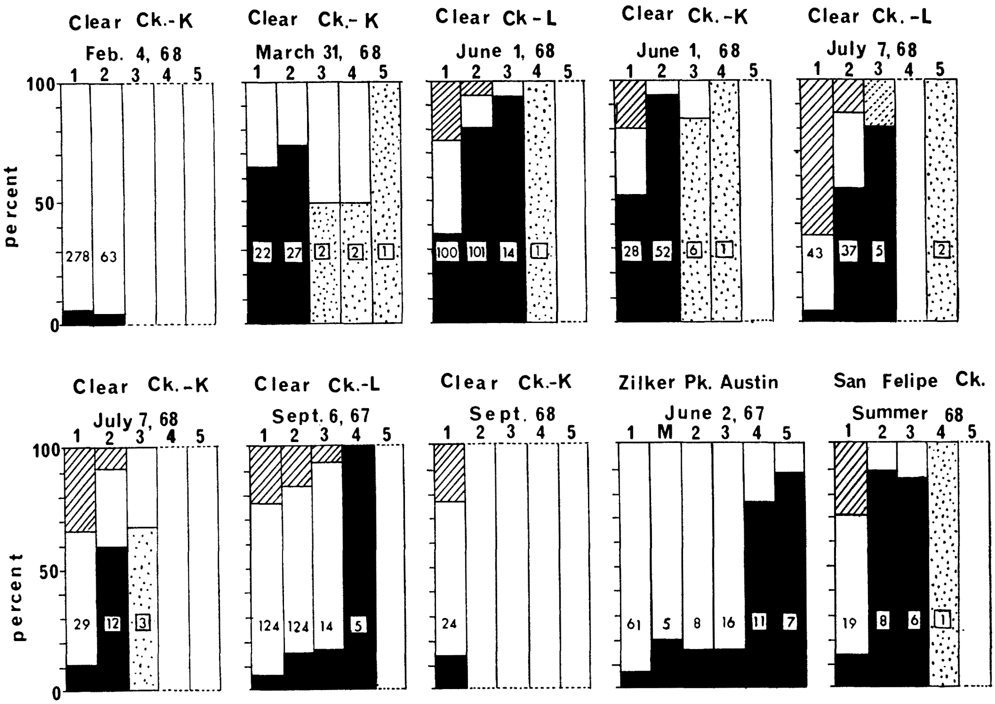

Fig. 7. Distribution of the percentage of G. *affinis* females with eggs (solid bars) and young (crosshatched bars) according to different intensities in the expression of their anal spots (see Fig. 1). Those females which have either no eggs or young, or have only small embryos, are represented by the white (hollow) bars. Each letter after a Clear Creek sample indicates a collecting station used in a study by Hubbs (1971). The number in each column indicates the number of preserved females in each category of anal spot development. Stippling refers to categories where the number of females possessing eggs were too few to indicate their percentage distribution within that category of anal spot expression. Dotted cross-hatching refers to categories where there were too few females with large embryos to indicate their percentage distribution within that category of anal spot expression. In the Zilker Park sample "M" indicates females with a few scattered melanophores in the genital region.

of relaxation or contraction of the sphincter muscles of the anal or urogential openings. In some species such as *G. geiseri*, the tissue external to the peritoneum is more transparent, making the dark coloration visible through these openings quite conspicuous (Figs. 5 and 6). This source of pigment might assist or supplement the presumed attractiveness of the anal spot to males. If male *Gambusia* see the genital region of females as a pattern similar to that described here, they probably orient to the conspicuous dark pigment on a white background in the genital region rather than the transparent tissue of the external genitalia of females. Since such tissue becomes opaque during preservation (Figs. 5 and 6), live specimens must be used in addition to preserved specimens to determine the patterns and intensities of ventral pigmentation.

Occurrences of anal spots on female *Gambusia* can be classified into three categories: those species which never possess anal spots; those in which anal spots vary in their expression according to the ovarian cycle; and those in which anal spots are present but do not fluctuate greatly in size or intensity during the ovarian cycle.

Cyclically Expressed Anal Spots.—Although *Gambusia affinis* is usually diagnosed as not having anal spots (Hubbs, 1959), anal spots were observed to vary in intensity during the ovarian cycle in individuals from widely scattered localities. When anal spots were coded (Fig. 1) and plotted in relation to whether the females contained eggs, embryos, or young (Fig. 7), the samples used from Clear Creek, Texas, exhibited seasonal variation. For example, female *G. affinis* captured at

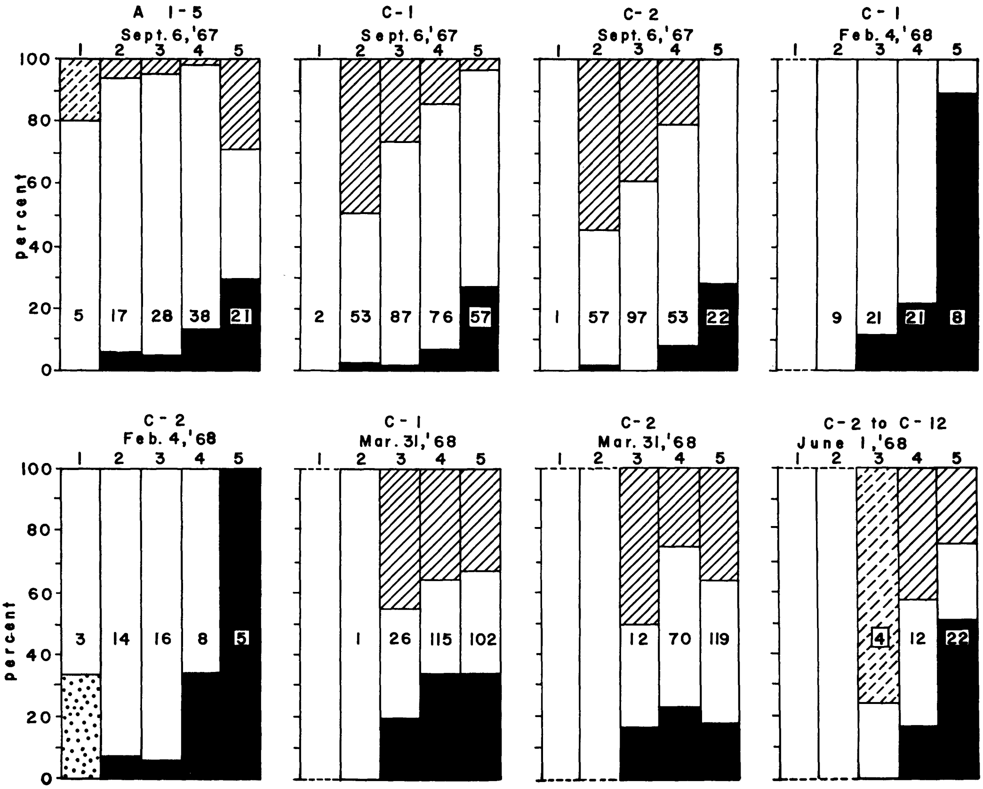

Fig. 8. Distribution of the percentage of *G. heterochir* females with eggs and embryos according to different intensities in the expression of their anal spots. See Fig. 7 for explanation.

Clear Creek during February did not possess young (Hubbs, 1971) and did not have dark anal spots (codes 3–5 in Fig. 7). Many females captured during June, July, and September, however, had anal spots or young. In addition, females with anal spots (codes 4 and 5) possessed eggs more frequently than those without anal spots (code 1). One would expect females with eggs ready for fertilization are more likely to advertise their presence to males. Contrastingly, females carrying large embryos ready for birth should be in least need of advertising their presence to males and predators and such females seldom had extensive development of anal spots (codes 1 and 2 in Fig. 7). Laboratory observations indicate that many females with anal spots contain sperm from copulations during earlier ovarian cycles. Anal spots in *G. affinis* were confined to the genital area but a few individuals from several localities were observed with black pigment over the entire abdominal area similar to that shown for *G. heterochir* in Fig. 6-f. A few populations in the Rio Grande drainage possessed females with a small amount of permanent dark pigment (code 1, Fig. 1) near the base of the third anal fin ray.

All females of *G. heterochir* (Figs. 1, 6, 8 and 9) had at least some evidence of an anal spot (Hubbs, 1959). Most females developed conspicuous anal spots (codes 3, 4 and 5). Those with the darkest anal spots (code 5) were much more likely to contain eggs than females with less conspicuous spots (Figs. 8 and 9). Small, pale spots (codes 2 and 3) usually occurred on females containing young. Because several technicians contributed to the data in Figs. 8 and 9, the variation between samples probably resulted in part from slightly different interpretations of color codes employed by individual observers. Some samples (March, 1968; A_1-A_5 and B_1-B_2 of July, 1968) had females assigned to higher code values so that an association of dark and large spots on females

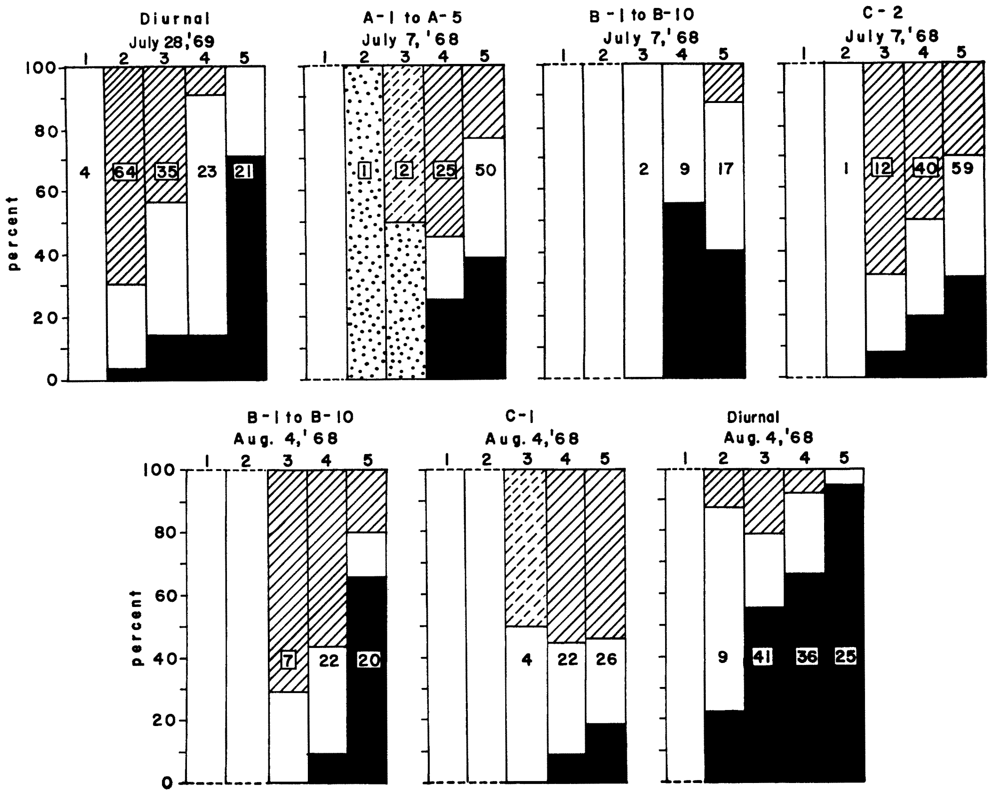

Fig. 9. Distribution of the percentage of *G. heterochir* females with eggs and embryos according to different intensities in the expression of their anal spots. See Fig. 7 for explanation.

with eggs could not be made; however, 12 other samples supported while none contradicted this association. Although samples taken in winter (February) have a few females with eggs and anal spots, they have none with young, indicating winter reduction of reproductive activity (Hubbs, 1971).

The rare *G. georgei* has anal spots similar to that usually found on *G. affinis* from Texas. Of the samples available for study, only one contained several females with large anal spots (Fig. 10) and each of these females possessed only eggs.

Very few collections of *G. geiseri* were observed to have anal spots (Figs. 1 and 10) and when present, their spots were inconspicuous and consisted only of thin rows of melanophores on each side of the urogenital opening. Eggs were present in all specimens of *G. geiseri* possessing these melanophores. Because dark pigment is also conspicuous in the anal opening, urogenital opening and gravid spots, it might play a role in the courtship of *G. geiseri* similar to that for the anal spots of other species, but these additional sources of dark pigment are not more prominent when eggs are present than when young are preesnt.

In contrast to the preceding species possessing crescentic anal spots on either side of the urogenital opening (Fig. 1), mature females of *G. amistadensis* posses a median spot between the urogenital and anal openings. To observe the condition of their ovaries in relation to anal pigment, 12 preserved female paratypes with large anal spots were examined and 10 of these possessed eggs while the other two had small embryos. Fourteen additional females judged to have smaller and less intensely colored anal pigment were examined; 10 had large young while the remaining four had medium-sized embryos. Apparently *G. amistadensis* also possesses anal spots which vary with the ovarian cycle.

Gambusia hurtadoi probably varies in the

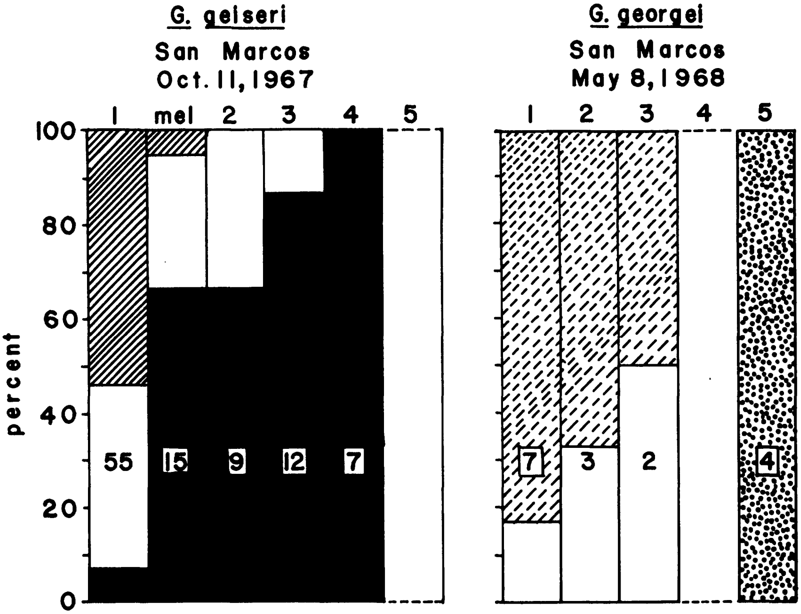

Fig. 10. Distribution of the percentage of G. *geiseri* and G. *georgei* females with eggs and embryos according to different intensities in the expression of their anal spots. See Fig. 7 for explanation.

intensity of expression of anal spots similar to the pattern described for G. *amistadensis* but large, freshly preserved samples from nature were not examined to establish this relationship. Although G. *hurtadoi* is generally characterized as having a median anal spot (Hubbs and Springer, 1957), a small percentage of the mature and healthy live females did not possess them. Others had small and large anal spots similar to those of G. *amistadensis*.

Because anal spots in some species of *Gambusia* were most prominent during that part of the ovarian cycle when eggs are present, it is logical to assume that anal spots in these species attract males to females at a time when sperm is most needed to fertilize eggs.

Anal Spots Not Varying with Ovarian Cycle. —Females of several species of *Gambusia* have permanent anal spots varying little during the ovarian cycle, but further detailed study could show subtle fluctuation of these spots. Small median anal spots were observed on most females of G. *gaigei*, G. *regani*, G. *marshi*, G. *panuco*, G. *krumholzi* and G. *caymanensis* (Figs. 5-C,D; and 6-A). *Gambusia nobilis* females had conspicuous crescentic spots extending around the urogenital opening (Fig. 1) and the base of the anal fin rays. Preserved females of G. *atrora* and G. *nicararguensis* had smaller crescentic anal spots Some collections of G. *aurata* had females with tiny dark anal spots between the urogenital sinus and anus, but in other collections this pigment was absent. The source for this variation is unknown.

Observations of Live Females

To test variation of anal spots with female ovarian cycles, I isolated five female G. *heterochir* in individual aquaria and recorded the presence or absence of anal spots at intervals between successive broods. Thirty-seven observations were made on these females during the first eight days after the birth of their brood; during the remainder of the period before the birth of their next brood, they were observed 31 times. Intervals between broods ranged from 30 to 33 days. For the first eight days intense spots were observed 12 times, while only twice were dark spots observed during the remainder of the cycle. Assuming no difference in the proportion of observations with dark spots throughout the ovarian cycle, a chi-square of

7.31 (probability less than .01) was obtained. Both intense and weak anal spots were recorded 20 times in the first eight days but only nine times during the remainder of the ovarian cycle. This distribution represents a chi-square value of 4.65 (probability less than .05). Therefore, anal spots predominated most during the early stages of the ovarian cycle when presumably eggs were being formed.

Factors other than those associated with ovarian cycles can alter anal spot intensity in some *Gambusia*. For instance, females of *G. heterochir* in aquaria usually had less intense anal spots than preserved females (Fig. 6), and perhaps less dark pigment than those in nature. Females can control the intensity of their anal spots as was shown by a series of experiments testing male preference for females with dark versus pale anal spots. In one series, a female possessed a dark anal spot at the start of an experiment but this spot vanished when I removed a partition from her aquarium. This phenomenon occurred with the same female on each of the several successive days that the experiment was repeated. I also observed anal spots to disappear in some species of *Gambusia* during unfavorable conditions. For example, all mature females of *G. krumholzi* and *G. regani* in aquaria stocks as well as most females in preserved collections, had median anal spots. When stocks of these species were introduced into artificial ponds outdoors at Austin, Texas, during the summer of 1970, *G. regani* soon overpopulated their pond and became emaciated. When the weather became hot and the water temperature reached 34 to 36 C, most females of both species lacked anal spots.

Because of the sources of variability described above and the difficulty of controlling the intensity of anal spots in some experiments, the results from tests of mate preference by males for conspecific females with or without anal spots are not reported here; however, Carlson (1969) and Peden (1970) present data indicating that males prefer females with darker and larger anal spots.

Male Courtship of Artificial Models

It quickly became evident, after a few trials, that male *Gambusia* were unlikely to thrust toward models lacking dark anal spots although they frequently thrusted when such spots were present. Whenever the anal spot was knocked off the model by a thrusting male, all males in the aquarium usually ceased thrusting until the anal spot was replaced.

Males of certain species of *Gambusia* rarely thrusted at artificial models. For instance, males of *G. vittata*, whose females do not have anal spots, left only one imprint on a model during dozens of periods of experimental tests. Male *G. geiseri*, *G senilis* and *G. atrora* readily approached models but rarely left gonopodial imprints in the shortening. Although imprints were frequently obtained from thrusts by *G. affinis*, *G. aurata*, *G. georgei*, *G. gaigei*, *G. heterochir*, *G. hurtadoi*, *G. regani*, and *G. marshi*, it is hazardous to compare differences in the frequency of thrusting by species towards models because the motivational factors that affect thrusting are not adequately known. Accordingly, only those events which occur after a male has been motivated to thrust must be considered here in experiments using models.

Because male *G. heterochir* occasionally left clusters of spermatophores in the hole made by their gonopodium in the shortening, the motor pattern of these thrusts toward models is probably very similar to the thrusting behavior made by males toward live females.

The location of anal spots was found to influence the orientation of gonopodial thrusts by male *Gambusia* (Fig. 4). For example, when models were suspended and stationary in an aquarium, male *G. aurata* which had previous experience with conspecific females, thrusted at whichever end of the model had the anal spot. When the spot was in the usual anal region, males made 17 thrusts from the normal posterior direction (direction indicated by arrow in bar graph of Fig. 4). When the spot was placed at the gill isthmus, only one thrust was made at the anal area, but 33 thrusts were made towards the head region and of the latter, 25 were made by males approaching from the anterior direction. Obviously male *G. aurata* oriented towards the dark pigment. Although males of other species were not tested in this manner, the experiments about to be described suggest they would probably thrust toward the head if the spot were placed there.

TABLE 1. DISTRIBUTION OF GONOPODIAL IMPRINTS MADE BY MALE *Gambusia* WHEN AN ANTERIOR PLACED ANAL SPOT WAS USED. See Fig. 3 for anal spot type.

	Species																											
	affinis spot type				*aurata* spot type	*heterochir* spot type				*gaigei* spot type				*hurtadoi* spot type	*krumholzi* spot type	*geiseri* spot type	*georgei* spot type				*amistadensis* spot type	*nobilis* spot type			*marshi* spot type			*regani* spot type
	A	E	H	S*	A	A	E	H	S*	A	E	H	S*	A	A	A	A	E	H	S*	A	A	H	S*	A	H	S*	A
Number of times gonopodium hit shortening anterior to spot	0	0	0	0	1	1	1	0	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	3	0	3	5
Percent**				0	7				3				0	0						0				0			8	38
Number of times gonopodium hit anal spot	0	5	2	7	4	16	4	8	28	6	1	7	14	4	0	0	2	3	0	5	0	2	2	4	16	4	20	4
Percent				14	26				35				37	24						20				33			53	31
Number of times gonopodium hit anterior half of shortening	9	19	1	29	6	11	8	8	27	15	2	0	17	11	3	2	3	6	2	11	1	2	1	3	9	0	9	3
Percent				58	41				34				45	65						46				25			24	23
Number of times gonopodium hit posterior half of shortening area	5	7	2	14	4	17	5	0	22	5	2	0	7	2	0	0	0	6	2	8	0	2	3	5	6	0	6	1
Percent				28	26				28				18	11						34				42			15	8

*S = Sum for that category.
** Indicates the percentage of all gonopodial imprints directed toward that area of shortening by each species of male.

TABLE 2. DISTRIBUTION OF GONOPODIAL IMPRINTS MADE ON SHORTENING BY MALE *Gambusia* WHEN A POSTERIORLY PLACED ANAL SPOT WAS PRESENT. See Fig. 3 for Anal Spot Type. See Table 1 for explanation.

	Species						
	affinis spot type		*heterochir* spot type		*gaigei* spot type		*regani* spot type
	C	F	F	J	C	F	C
Number of times gonopodium hit shortening anterior to spot	0	0	3	0	0	0	3
Number of times gonopodium hit anal spot	1	6	1	1	1	0	3
Number of times gonopodium hit posterior half of shortening	1	8	0	0	3	1	3

When presented with models possessing spots in the anal area, male *Gambusia* usually approached from the posterior direction until they reached and then thrusted at some irregularity (usually the anal spot) on the ventral surface of the model. They were not particularly discriminating because male *G. affinis, G. georgei, G. gaigei,* and *G. heterochir* occasionally thrusted at the thread which had been tied around the caudal peduncle of the model. Some males also thrusted at blemishes along the mid-ventral line or when excited, occasionally at debris in the water and air bubbles at the surface. In experiments when spots were painted on models in the position where gravid spots occur, male *Gambusia* never thrusted at them or any spots which were placed away from the ventral midline. Black models with white anal spots were also presented but were ignored by males.

Although a variety of anal spots (Tables 1–4) were presented to male *Gambusia*, only a few gonopodial imprints were obtained in any one test, and consequently, only when data are accumulated from several tests can general conclusions be made. Most noticeably, male *Gambusia* were relatively inaccurate because more than 50% of the thrusts did not leave imprints and were consequently not recorded. When imprints were left, they were scattered in the shortening around the anal spot and were not directed consistently to any one area (Tables 1–4).

TABLE 3. DISTRIBUTION OF GONOPODIAL IMPRINTS MADE ON SHORTENING BY MALE *Gambusia* WHEN A CENTRALLY PLACED ANAL SPOT WAS PRESENT. See Fig. 3 for Anal Spot Type.

	Species									
	affinis spot type	*aurata* spot type		*heterochir* spot type		*geiseri* spot type	*georgei* spot type	*amistadensis* spot type	*nobilis* spot type	*regani* spot type
	B	B	I	B	I	B	B	B	B	B
Number of times gonopodium hit shortening anterior to spot	0	0	0	0	0	1	0	0	0	3
Number of times gonopodium hit spot	3	1	2	0	1	0	2	1	0	3
Number of times gonopodium hit shortening posterior to spot	2	5	0	2	0	0	0	3	2	4

TABLE 4. DISTRIBUTION OF GONOPODIAL IMPRINTS MADE ON SHORTENING BY MALE *Gambusia* WHEN TWO ANAL SPOTS WERE PRESENT. See Fig. 3 for Anal Spot Types.

	Species				
	affinis spot type G	*heterochir* spot type K	*gaigei* spot type K	*geiseri* spot type G	*georgei* spot type K
Number of times gonopodial imprint anterior to spots	0	0	0	0	0
Number of times gonopodium hit anterior spot	0	1	2	0	0
Number of times gonopodium hit shortening in anterior half	0	0	0	0	0
Number of times gonopodium hit posterior spot	2	8	14	0	1
Number of times gonopodium hit shortening in posterior half	3	2	0	1	1

Most commonly, *Gambusia* males did not thrust anterior to, but rather directly at or posterior to, the spot. When anal spots were placed more posteriorly in the area of the shortening (Table 2), only a few thrusts were recorded since most thrusts were probably directed too far posteriorly to leave an imprint. When both an anterior and posterior spot were provided, imprints were usually left near the posterior spot (Table 4).

To determine if the physical presence of the anal fin impeded thrusts by males, a clear plastic anal fin was glued to the models (spot types E, F, and G of Fig. 3 in Tables 1, 2, and 4). When the anal spot was placed next to the anal fin base (F of Fig. 3 in Table 2), *G. affinis* males still thrusted between the anal fin base and the anal spot, but the few thrusts obtained from *G. heterochir* were directed more anteriorly.

In several tests, crescents (Figs. 3-H, I, and K) were presented instead of a median spot; however, no major differences were noted in the relative positions of the few gonopodial imprints which were obtained. Males thrusted at, between or posterior to, but seldom anterior to, crescents (Tables 1 and 3).

DISCUSSION

Sexually receptive motor patterns by female *Poecilia* toward conspecific courtship patterns by males were described by Liley (1966). The cyclic variation of the anal spot during oögenesis in some species of *Gambusia* is apparently a form of receptive behavior that facilitates male courtship. Similar pigmentary changes correlated with the ovarian cycle have been observed in females of *Neotoca* of the related family Goodiidae (Mendoza, 1940). Whether females of *Gambusia* have distinct motor patterns of receptive behavior toward conspecific patterns of male courtship similar to those found in *Poecilia* by Liley (1966) remains to be documented. Carlson (1969) demonstrated that females of *G. affinis* had a greater frequency of anal spots shortly after the birth of their broods and were courted more frequently during this period, but his statement that "It has often been surmised that the anal spot is present during female receptivity and is a target for male thrusting" was not documented. Most importantly, the data used in his study on female receptivity were subjective because his definition of receptivity is interrelated with, and partly defined by, the thrusting activity of males. Therefore, it is difficult to analyze how much of the thrusting response by males was caused by the attractiveness of anal spots or by the motor patterns of the females. Although Liley (1966) used female *Poecilia* which were at a stage of the ovarian cycle during which they were sexually receptive to male courtship, many other studies on mate preference in poeciliids have not stated whether females at a similar stage in the ovarian cycle were used. Obviously the use of females with anal spots of different sizes and intensities of coloration could affect male preference.

Because models without anal spots seldom induced thrusting behavior by males, the experiments in this study using models with anal spots strongly indicated dark pigment is important in motivating or triggering this behavior. After males were motivated to thrust, the dark pigment facilitated the orientation of thrust. Since the genital opening of most species of *Gambusia* is surrounded by or is posterior to the anal spot, we would expect the gonopodial imprints from males on the artificial models to be also at or posterior to, rather than anterior to, this spot.

ACKNOWLEDGMENTS

This paper is extracted from a dissertation (Peden, 1970) submitted to the University of Texas at Austin in partial fulfillment of the requirements for the degree of Doctor of Philosophy. I am particularly in debt to Clark Hubbs who in addition to contributing helpful advice, provided me with the facilities to pursue this problem. Jeanne Davis, Paulette Setzer, Sharon Wehner, W. S. Birkhead, W. F. Blair, J. Bogart, J. W. Gerald, E. L. Lundelius, M. Wheeler, T. Buchanan, C. Langely, G. Powell, M. Stevenson, B. Trent, J. Wiersema and D. Yardley also contributed valuable discussion, criticism, and/or assisted in the collection of live material. The National Museum of Natural Sciences, National Museum of Canada provided some facilities and support during the final preparation of this report. My wife Jana assisted in the typing and drew several of the illustrations.

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