

Courtship Behavior of the Oriental Fruit Moth (*Grapholitha molesta*)¹: Experimental Analysis and Consideration of the Role of Sexual Selection in the Evolution of Courtship Pheromones in the Lepidoptera²

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ABSTRACT

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The courtship behavior of *Grapholitha molesta* (Busck) (Oriental fruit moth) males, as analyzed by means of conditional probability matrix techniques, consists of a relatively stereotyped sequence of behaviors culminating in an elaborate hairpencil display directed toward and performed 1 or 2 cm from the female. The display is comprised of multiple rhythmic extrusions and retractions of the white hairpencil organs and claspers at the end of the abdomen, accompanied by corresponding 45 and 90 cm/sec "puffs" of wind generated by the vibrating wings and directed toward the female. The stimuli contained in the display, primarily chemical and anemo-tactile (wind movement), attract the female, who walks to the hairpencils, where contact with the end of the abdomen causes the female to cease walking. The tactile stimulus from the female causes the male to turn quickly and attempt copulation. The female again walks forward, with the wing nearest the male raised, and copulation follows. The visual stimulus of the calling female acts in conjunction with female-emitted pheromone components to release the sequence of male courtship behavior. Visual feedback from the attracted female moving toward the male causes the form of the display to be altered, making the 1st extrusion longer. Successful courtship sequences last ca. 1.5 sec.

The male hairpencils and courtship behavior of this species are hypothesized to have evolved primarily by means of sexual selection involving "female choice", in which a female preference and preferred male trait become linked as a result of the mating advantage conferred upon male offspring of discriminating females. Male courtship pheromones of other Lepidoptera and "coyness" in females also may have evolved by this mechanism. The hairpencil display is viewed as a form of clasper extension behavior that has become "ritualized" through the process of sexual selection.

Many males of the Lepidoptera possess elaborate brush organs or hairpencils, which are kept in pockets or pouches on various areas of the body until courtship, when they are everted in the presence of a female. While most of the courtship pheromones in Lepidoptera described to date elicit in females such responses as flight cessation, quiescence or abdominal extension (see Birch 1974 for review), orientation responses by females to courtship pheromones rarely have been described. We report here that males of a tortricid pest of peaches and apples, the Oriental fruit moth, *Grapholitha molesta* (Busck), give a stereotyped elaborate hairpencil display whose chemical and wind stimuli cause locomotion and attraction of a female to the hairpencils from up to 2 cm away. We propose that the display represents a form of ritualized clasper extension which evolved via "female choice" sexual selection (Fisher 1958).

George (1965) first described the eversion but not the function of "scent-pencils" of male *G. molesta* exposed to pheromone from a neighboring container of females. Roelofs et al (1969) identified the major component of the *G. molesta* female sex pheromone, and it was later found that additional components were necessary for optimal catch of males in sticky traps (Roelofs and Cardé 1974, Beroza et al. 1973). Some of these components at close range caused increases in the amount of landing, wing fanning while walking, and hairpencil display behavior, and the hairpencil extrusions appeared

to attract calling females from short distances (Cardé et al. 1975).

We present here a detailed analysis of the sequence of male courtship behavior, the female stimuli which release the male behaviors, and the stimuli contained in the male hairpencil display which affect female behavior.

Materials and Methods

Rearing

Grapholitha molesta adults came from a laboratory colony maintained at Michigan State since Oct. 1975 on apples on a 16:8 light: dark photoperiod regime at 25°C. Feral adults were added to the mating stock when available each summer.

Description and Sequence Analysis of Courtship Behavior

We recorded courtship behavior in the laboratory by placing a calling female (emitting pheromone) onto an open horizontal observation platform of black paper or cloth and then releasing, downwind, a male from a glass vial. The ensuing behavior was recorded on a Sony AVC-3540 videorecorder through a Sun III time base generator to number the frames each 1/30 sec. We played back the tapes on a Concord VTR 820 deck through a time base decoder onto a television monitor and transcribed the behavior frame by frame onto paper for analysis of sequence and temporal patterns. Durations of hairpencil extrusion and retraction were measured from the transcribed data using the first and last visible appearances of white hairpencils as markers for

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the start and finish of an extrusion and the interval between (no white hairpencils visible) as the duration of retraction.

We used the sequence analysis techniques that others have used for similar studies (e.g., Baerends et al. 1955, Halliday 1975). Frequencies of transitions from one behavior to another were first tabulated from the transcribed recordings and then consolidated into a transition probability matrix using only first-order transitions. Self-transitions (i.e., when, after a predetermined time interval, the animal remains in the same behavioral state) were not included in these matrices. In sequence analysis, the importance of transitions between behaviors can be obscured if self-transitions are included, as the latter usually occur within long "bouts" of one behavior and are therefore more numerous (Slater and Ollason 1973). Hence, the final matrices and resulting flow diagrams (Fig. 4) are of *conditional* probabilities, i.e., the probability of a particular transition occurring given a change in behavioral state will occur.

Effect on Male Behavior of Visual Stimulus at Pheromone Source

We constructed a special platform (Fig. 1) and placed it in a laboratory wind tunnel (described in Cardé and Hagaman 1979) having a photophase (daytime) lighting

intensity of 150 lux, to test males' visual response to calling females. The platform was a thin sheet of white polystyrene plastic 23.5 × 24.5 × 0.05 cm covered by a black cloth, with a 1 mm diam hole through the center of only the plastic into which the tip of a Pasteur pipette was placed. A rubber septum was impregnated with 4 components of the female-produced sex pheromone (Cardé et al. 1979): 9.5 μg (*Z*)-8-dodecenyl acetate, 0.5 μg (*E*)-8-dodecenyl acetate, 0.1 μg (*Z*)-8-dodecen-1-ol, and 10 μg *n*-dodecanol, all pipetted in 10 μl from a hexane solution. From gas chromatography, we determined that all compounds were greater than 99% pure; the acetates contained less than 0.05% 12-carbon alcohols and the (*Z*)-8-dodecen-1-ol no detectable amounts of the (*E*) isomer. We placed the septum in a glass vessel through which filtered air was blown at the rate of 0.4 ml/sec, and the pheromone-laden air passed up the pipette and through the cloth on top of the platform; thus no visual cues were present on the black cloth surface.

Wind in the tunnel at a velocity of 0.7 m/sec blew the pheromone to the downwind end (where it was exhausted from the building), and we released males individually within the pheromone plume from an 11 × 7 cm diam screen cylinder from which males flew to the platform, landed, and approached the chemical source by fanning their wings while walking.

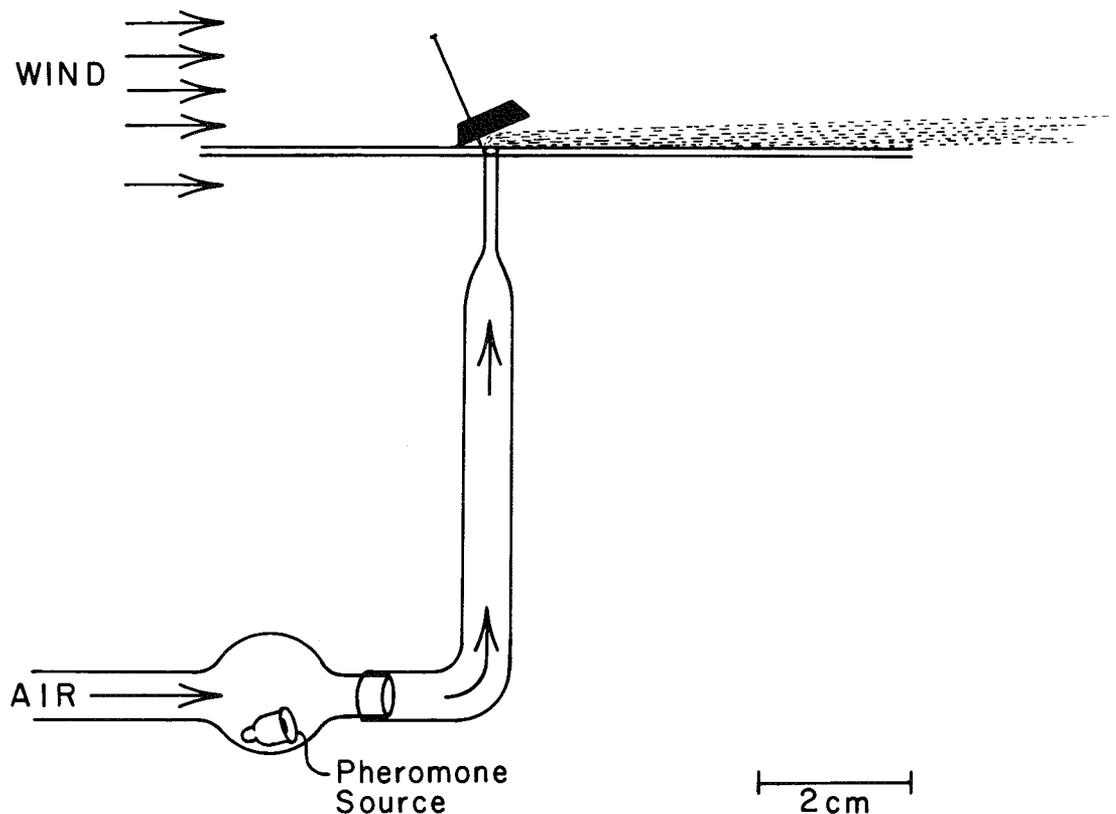


FIG. 1.—Diagram of the lateral view of the observation platform used for presenting *G. molesta* sex pheromone both with and without a visual stimulus to males flying upwind from the right. Pheromone-laden air was blown through the tip of the pipette through a layer of black cloth covering the plastic platform surface and traveled downwind in the wind tunnel at a rate of 0.7 m/sec.

We used 3 different 3-dimensional visual models to test male courtship response: a dead male washed with acetone and pinned to the platform over the hole, a gray piece of construction paper (7 mm long, 3 mm high, 3 mm wide at the posterior end) (Fig. 10) folded in half and pinned over the hole, and a rubber septum (Fig. 7) (Arthur Thomas Co.) 15×9 mm diam at the widest end. Models were tested against the control situation of no visual stimulus present, and the number of males in which hairpencil display was elicited was recorded.

Effect of Spatial Separation of Visual and Chemical Stimuli

We modified the platform described above to test the effect of moving a visual model away from the chemical source. We removed the black cloth and drew in pencil on the plastic to divide the surface into quadrants: upwind, downwind, left, and right. A small piece of white tape was placed loosely on the center of the platform to conceal the hole and the tip of the Pasteur pipette, as preliminary trials had indicated that these visual cues could increase the frequency of hairpencil display behavior. The gray paper model described above was pinned 1 cm from the pheromone source in one of the quadrants or on top of the source or removed completely, using a completely randomized design. Males were released at the downwind end of the tunnel and their behavior upon landing on the platform was recorded on videotape. For each male, the number of hairpencil displays and percentage of time spent in each of the quadrants both away from (>0.5 cm) and at (≤ 0.5 cm) the chemical source were calculated after a frame-by-frame analysis of the tapes. All displays, regardless of their direction, were scored for each model location. The percentages of time spent in quadrants for each individual were transformed to $\arcsin \sqrt{x}$ and the data were submitted to a 2-way analysis of variance for unequal replication. Means were compared using the LSD test. Mean numbers of hairpencil displays per male were compared by the LSD test after a one-way analysis of variance for unequal replication.

Effect of Female Movement on the Form of Male Hairpencil Displays

Videotape recordings revealed a positive correlation between the duration of the 1st hairpencil extrusion of the 1st display and the success of males in attracting females. To test whether female movement toward the male was causing longer extrusion durations, a rubber septum was impregnated with the pheromone mixture described earlier and affixed from beneath by an insect pin to the observation platform. When a male began his display we either rotated the model rapidly 180° back and forth by twirling the pin from beneath, or left it stationary. The behavior was video-recorded and the extrusion durations measured later frame-by-frame.

Effect of Tactile Stimulus from Female after Attraction to Male Abdomen

Using the platform and gray paper model, the hairpencil display was evoked in males landing on the platform after flying upwind in the tunnel. We tried to touch males' abdominal tips with a camel's-hair brush dusted with Day-Glo® rocket red powder during their first dis-

play at the model, and observed the subsequent behavior. The males were recaptured and the hairpencils checked using ultraviolet light for evidence of transfer of the powder. Transfer of powder was considered proof that contact with the abdomen had actually been made by the brush. We ran controls at random, holding the brush near the abdomen, but trying not to touch it.

Stimuli from Male During Hairpencil Display Affecting Female Courtship Behavior

The male hairpencil display contains many types of stimuli of potential importance in attracting the female to the male. Three stimuli thought to be important are: (1) chemicals emanating from the hairpencil surfaces; (2) visual stimuli presented by a male's white hairpencils; and (3) the mechanical stimulation from wind generated by the male's vibrating wings. We investigated the relative importance of these 3 stimuli in the following way.

We used 2 groups of calling females, 1 group sham-operated, and the other "blinded" by the following procedure. After being chilled for a few minutes in a freezer, females were picked up by their wings with a pair of forceps and a small amount of white water-soluble glue was applied to both compound eyes with an insect pin until no ommatidia were visible. We sham-operated females by placing a drop of glue between, but not covering, the compound eyes. During the normal calling period, we tested individual females from both groups in a randomized complete block design for their response to a hairpencil extract chemical stimulus, wind stimulus, chemical and wind stimuli combined, and respective solvent blank controls. The response of blinded vs. sham-operated females to the extract- or solvent-impregnated white filter paper presented against the black background was considered a measure of the importance of vision in female orientation to hairpencils.

A female, either blinded or sham-operated, was placed on the horizontal cloth-covered platform and allowed to adjust to the new conditions for ca. 1 min. Then 1 of the 4 treatments described below was directed toward the female from 1 cm directly upwind and the resulting behavioral response recorded.

We made hairpencil extract by taking frozen males, excising the terminal 3 abdominal segments including hairpencils, and extracting these in re-distilled methylene chloride. Approximately 5 male equivalents (50 μ l) were pipetted onto a piece of folded filter paper attached to and protruding 1 cm from the tip of a disposable pipette (1 mm ID). The solvent was allowed to evaporate for 20 sec and then the tip of the paper was placed 1 cm upwind of a calling female (ambient wind velocity of ca. 33 cm/sec). For another treatment, additional wind was added by blowing filtered air at a velocity of ca. 76 cm/sec (measured 1 cm from the tip) through the pipette, over the filter paper, and then onto the female 1 cm downwind of the filter paper. Solvent blank controls were made using 50 μ l of methylene chloride.

Measurement of Wind Velocity Generated by Male Wing Fanning During Hairpencil Display

To measure the velocity of wind generated posterior to a male during hairpencilling, we constructed an anemo-

meter consisting of a 6 cm long section of a single branch of a peacock feather suspended vertically from a horizontal bend on a 6 cm high piece of wire. The wire was affixed to the observation platform so that the feather hung just beside and behind the gray cardboard model pinned directly over the pheromone source (Fig. 11A). Males hairpencilling at the model caused the feather to be deflected backwards, and during playback analysis of videotapes made of these males, the deflection magnitudes were measured on the television screen for the periods when the hairpencils were fully extruded and retracted. Immediately after a session of taping male behavior, we also made recordings of feather deflections from a known velocity of air emanating from a tube (0.6 cm ID) placed 2 cm directly upwind of the feather. Wind velocities of 25, 51, 102, 152, and 254 cm/sec were used to deflect the feather, as first measured on a Hastings-Raydist model AB-27 anemometer, and the deflections measured on the television monitor. We then converted the magnitude of deflection caused by hairpencilling males to wind velocities in cm/sec by interpolation.

Measurement of Female Preference for Males with Intact Hairpencils

We first chilled males to immobility in a freezer ca. 6 h before lights-off, and while the male was gently grasped by the wings and middle of the abdomen with forceps, the end of the abdomen was exposed and dabbed with a drop of clear glue from an insect pin. Glued males were incapable of extruding their hairpencils. We handled sham-operated males identically except that we rubbed a clean insect pin 4 or 5 times across the abdominal tip; a 2nd sham-operated group consisted of males to whose pronota glue was applied.

To test female response to males from different groups, at 0.5–3 h before lights-off we placed an individual "glued" male onto the observation platform located in a laboratory exhaust hood. A calling female was then placed upwind of the male, and the ensuing hairpencil displays and female response were noted. After the male and female separated (usually after the female walked or flew away) we removed the male, returned the same female to the middle of the platform, and introduced a sham-operated male (from the 1st sham group) downwind of the female. Hairpencil displays and female response were recorded. To test the possibility that dried glue on the male's body was influencing female response, males from the 2nd sham-operated group, containing glue on their thoraces, were introduced downwind from naive calling females; the resulting behavior was observed and recorded.

Results

Description of the Courtship Sequence

Analysis of 49 courtship sequences revealed that a fairly fixed pattern of male and female behavior occurs. After the male walks towards the female from downwind, fanning his wings (Fig. 2A), the usual sequence is this: he (1) pauses briefly less than 1 cm from the calling female, facing her (Fig. 2B); (2) moves past her into a new quadrant, which usually brings him upwind

of her; (3) turns and points his abdomen's tip at her, thus facing away from her (Fig. 2C); (4) extrudes his hairpencils (Fig. 2D) which are located between the 7th and 8th abdominal segments and associated with the claspers. After the 1st extrusion, the female ceases calling and begins walking toward the male's abdomen tip (Fig. 2E, F). While the female approaches, the male extrudes and retracts his hairpencils 1 or 2 more times until the female touches his abdomen with her head and antennae (Fig. 2G), pausing momentarily. The contact causes the male to attempt copulation, first arching the tip of his abdomen even higher (Fig. 10B) and then abruptly whirling in a type of horizontal cartwheel while touching the female (Fig. 2H, 10C). During the male's attempted copulation, the female continues walking, but with the wing on the side of the male raised, possibly due to the force of his attempt (Fig. 2H); her abdomen is grasped by the male's claspers and copulation occurs. The male's wings rest on top of the female's (Fig. 2I).

Males not inducing females to make contact with their abdomen repeated the courtship sequence many times (Fig. 3) before finally attempting to copulate, usually unsuccessfully. As a result of the movement into new quadrants between hairpencil displays, males directed the displays toward the female from many different angles (Fig. 3); sometimes males only attracted females after 2 or 3 displays from different positions. Other unsuccessful males were those touching the female while moving past her either before or between displays, causing her to fly or walk rapidly away; these males remained wing fanning while walking ($N = 25$). Although these encounters were not included in further analyses of courtship behavior, they are important in showing that an "improper" performance of even the earliest stages of the courtship behavior can reduce the probability of mating.

Sequence Analysis of Male Courtship Behavior

As evidenced by the high conditional probabilities of proceeding only to the next step in the courtship sequence, male courtship behavior is fairly "stereotyped" (Fig. 4). For males both successful (Fig. 4A) and unsuccessful (Fig. 4B) in attracting females to their abdomens ($N = 27$ and 22, respectively), there is a high probability that once the 1st step in the sequence is taken the rest will follow. The probability of hairpencil retraction immediately following extrusion is so high that, for sequence analysis, little information is gained by keeping these as separate behaviors; they are presented separately in the diagrams, however, to make it clear that the hairpencils are repeatedly extruded and retracted.

The major difference between the behavior of males successful and unsuccessful in attracting females is seen following the hairpencil display. Successful males, i.e., those receiving the apparent "touch" on the abdomen from the female, have a high probability (0.84) of making a copulatory attempt following hairpencilling (Fig. 4A); for unsuccessful males the probability is low (0.12) (Fig. 4B). For successful males the attempt results in copulation 70% of the time. Rather than attempting copulation after hairpencilling, unsuccessful males have a high probability (0.79) of returning to an earlier step,

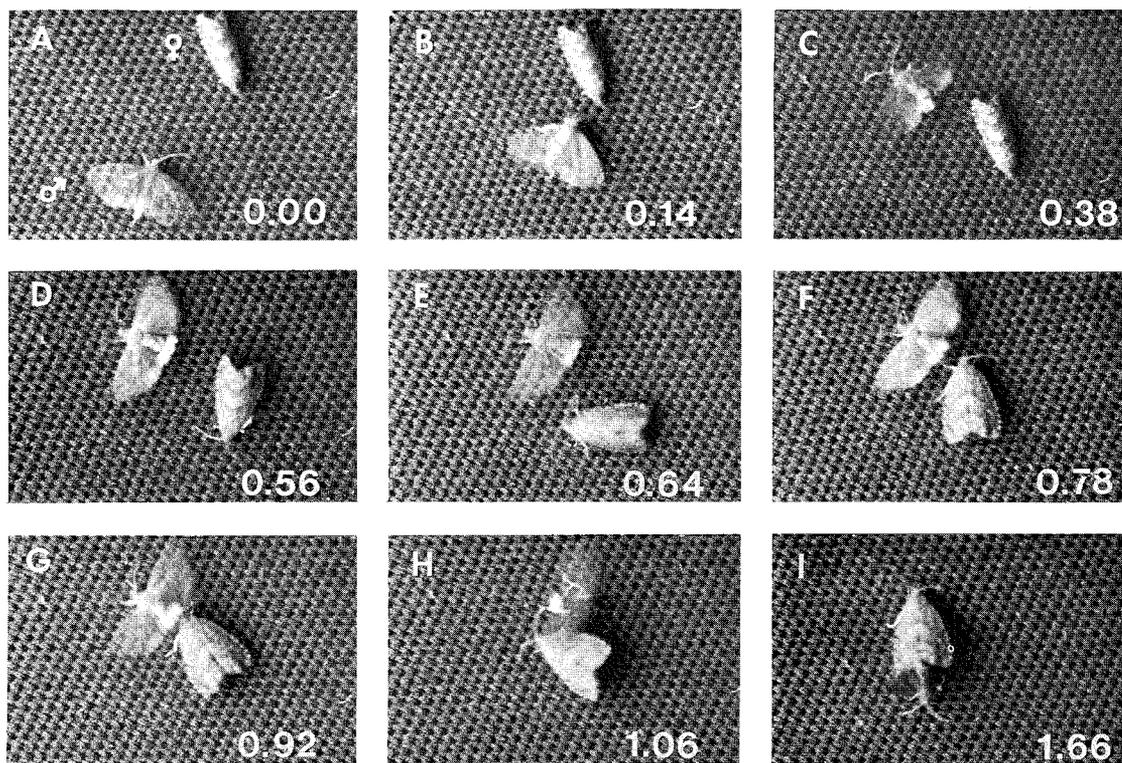


FIG. 2.—The sequence of events occurring during courtship and copulatory behavior: A) male approaching calling female from downwind by wing fanning while walking; B) male faces female; C) male has moved past female and faces away, female still stationary; D) male extrudes hairpencils, female begins walking toward male; E), F) male hairpencils remain extruded, female continues walking toward male; G) female about to make contact with abdomen; H) contact with abdomen made, male begins to attempt copulation; I) copulation, with male's wings about to settle on top of female's. Numbers in lower right hand corner indicate time elapsed in seconds. Wind direction is from top of picture to bottom.

facing the female, and then running through the sequence again. The high probability of "moving past the female" each time through the loop indicates that these males often move to a different side of the female during each successive display, possibly to help improve the "angle" or directionality to evoke a female response. With each successive display the number of hairpencil extrusions decreases from an initial mean of 3.8 during the 1st to 2.1 during the 5th display (Fig. 5), until a copulatory attempt is finally made. Such attempts are usually unsuccessful and the female flies or walks away. The male then has a high probability of returning all the way to wing fanning (0.92) either to follow the disturbed female or to "search" the area she has vacated. No behavioral steps preceding wing fanning are included in this analysis, but when the female departs, the male eventually stops wing fanning and returns to earlier behaviors such as flying downwind or sitting.

Temporal Analysis

The entire *G. molesta* courtship sequence occurs in a short time span, generally less than 1.5 sec from momentarily pausing and facing the female to successful copulation. For males whose displays attracted females, the mean time from facing the female to the beginning of hairpencil display was 0.31 sec (± 0.16 SD; $N = 18$). The mean duration of the interval beginning hairpencil

display to copulatory attempt was 1.04 sec (± 0.36 ; $N = 27$). The copulatory attempt (the quick whirl of a touched male) is difficult to observe on videotape and appears to have a duration no longer than 1/30 sec. Females took mean times of 0.36 sec (± 0.21) and 0.66 sec (± 0.36 ; $N = 27$) to walk to males' abdomens from the beginning of the 1st extrusion and from calling position, respectively. The mean duration of the male's 1st extrusion was longer when females were moving than when they were stationary—a significant temporal difference (Table 1). Within a particular display the first extrusion lasts longer than the last (Fig. 6), but this trend is exaggerated for the 1st display of males successfully attracting females (solid circles, Fig. 6). That both the number of extrusions per display (Fig. 5) and the duration of extrusions (Fig. 6) within a display decrease through time, may be a result of a number of factors such as sensory adaptation, habituation, fatigue of the efferent system, or a decrease in internal drive. We do not know the factors responsible for these changes in displays.

The correlation between success and extrusion duration could be caused by a number of factors: an innate difference in male hairpencil behavior conferring an advantage on those who keep their organs extruded longer; a difference in female stimuli releasing hairpencil display whereby females more likely to respond to the display also emit stimuli more likely to initiate a better dis-

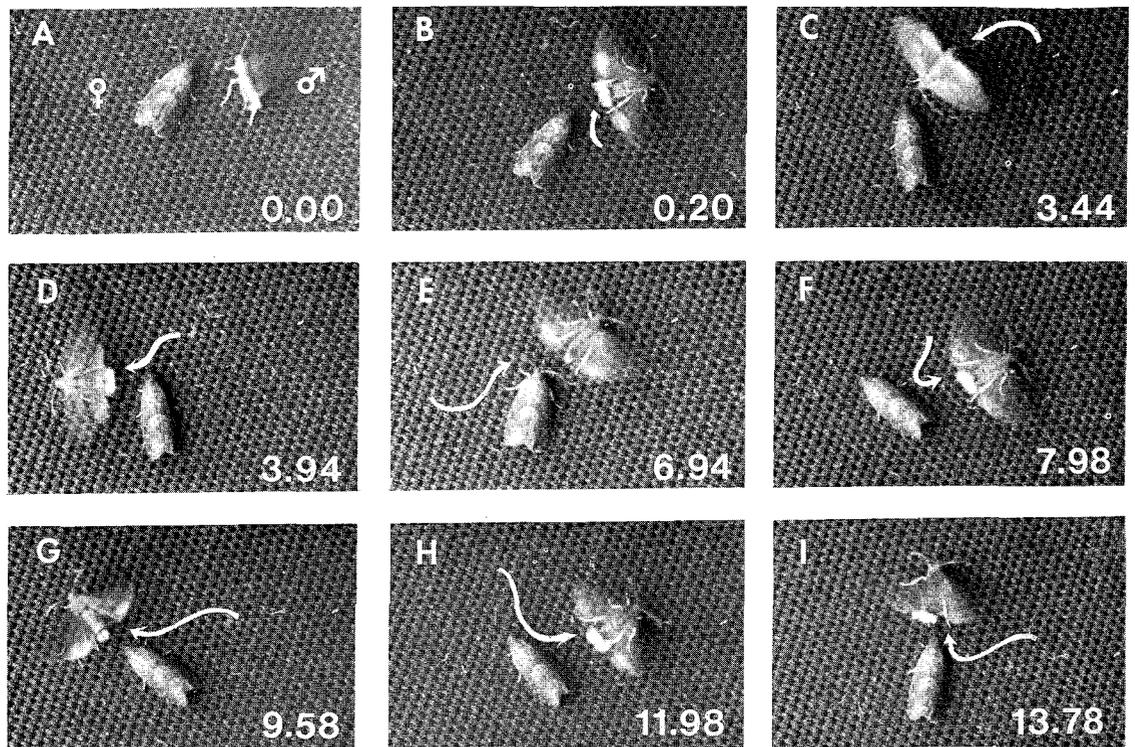


FIG. 3.—Male courtship behavior, consisting of 7 different hairpencil displays when the female does not make contact with the male's hairpencils. Arrows indicate the path taken by the male. In figures B, E, and H, the female is about to move toward the male but not touch his abdomen, and then ends up sitting in a new position in C, F, and I. A) male moves past female after approaching from downwind; B) male faces away and gives the hairpencil display consisting of 4 extrusions; C) male turns and faces female after finishing the display; D) male moves past female, faces away and gives hairpencil display; E) as in C and D; F) as in C and D; G) as in C and D, hairpencils beginning extrusion phase; H) as in C and D; I) as in C and D. Numbers in lower right hand corners indicate time elapsed in seconds.

Table 1.—Effect of female locomotion toward male upon mean hairpencil extrusion durations. Only males which eventually had females move toward them were used in this comparison. **, entry in same column significantly different according to the *t*-test ($P \leq 0.01$); NS, entry in same column not significantly different according to the *t*-test ($P > 0.05$).

	Mean duration of hairpencil extrusions (\pm SD)		
	1st extrusion	2nd extrusion	3rd extrusion
While female is moving toward male	0.54 \pm 0.31 sec** N = 22	0.36 \pm 0.61 sec NS N = 20	0.30 \pm 0.12 sec NS N = 23
While female is stationary	0.33 \pm 0.09 sec N = 20	0.29 \pm 0.12 sec N = 19	0.27 \pm 0.05 sec N = 22

play; or, males watch the female and adjust their display according to whether she approaches them or not. Results of a later experiment show that the last hypothesis is at least 1 of the causes of this display difference.

Effect of Visual Stimuli at Pheromone Source

The percentage of males giving the hairpencil display increased significantly when female models were at the

origin of the pheromone source (Table 2). Of males reaching the source by wing fanning while walking, 64% displayed at dead acetoned males, 47% at the gray paper model, and 52% at the rubber septum (Fig. 7). The controls elicited 13, 0, and 5% displays, respectively. Males in both groups not hairpencilling remained wing fanning while walking on top of either the chemical source or the models before sitting or flying back

Table 2.—The effect of the presence of visual models at the pheromone source upon frequency of hairpencil behavior; **, entry significantly different from the one immediately below it according to a $\chi^2 2 \times 2$ test of independence with Yates' correction ($P \leq 0.01$).

	No δ wing fanning at pheromone source	No. δ giving hairpencil display (%)
Dead male present	28	18 (64%)**
Dead male absent	32	4 (13%)
Paper model present	30	14 (47%)**
Paper model absent	30	0 (0%)
Rubber septum present	21	11 (52%)**
Rubber septum absent	21	1 (5%)

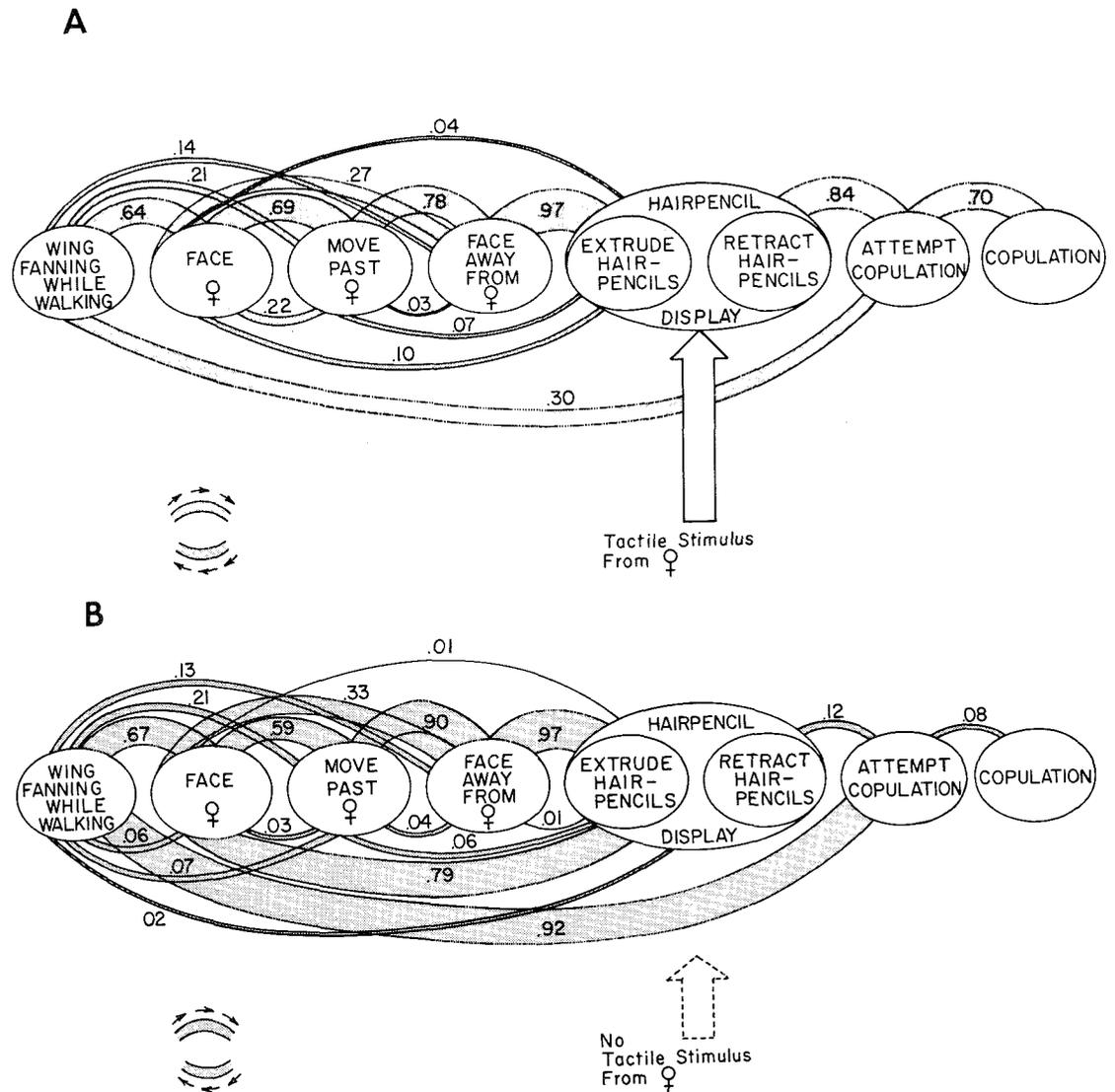


FIG. 4—Sequence of courtship behavior observed in *G. molesta* males both successful (A) (N = 27) and unsuccessful (B) (N = 22) in attracting females to their abdomens during hairpencil display. Decimal numbers and corresponding thicknesses of bands are the conditional probabilities of a particular transition occurring between 2 behaviors. Behavior flows from left to right in the upper bands and right to left in lower bands

downwind. Displaying males always faced away from, and thus directed their displays toward, the models or the source.

These results indicate that a visual stimulus in conjunction with the pheromone blend (Cardé et al 1979, Baker and Cardé, unpubl.) releases courtship behavior more frequently than the chemicals alone. Because effectiveness of the 3 models was not contrasted in a single experiment, comparisons between them cannot be made, but it is clear that each one was effective in eliciting the hairpencil display when combined with chemical stimuli. Their effect can be viewed in one way as causing a lowering of the chemical threshold for hairpencil display behavior; or, the visual and chemical stimuli can be viewed as acting together as an algebraic sum which sur-

passes the minimum value necessary for hairpencil display to occur. The latter explanation has been called the "heterogeneous summation" of stimuli (Lorenz 1950), and used to explain the many ways a behavioral response can sometimes be elicited by varying the relative stimulus intensities.

Effect of Separation of Visual and Chemical Stimuli

The gray paper model was most effective in releasing hairpencil behavior when it was placed directly upon the chemical source, less effective downwind or to the left, and no more effective than no model at all when it was upwind or to the right of the source (Fig. 8). No matter where the model was placed around the source, the percentages of time spent in a particular quadrant by males

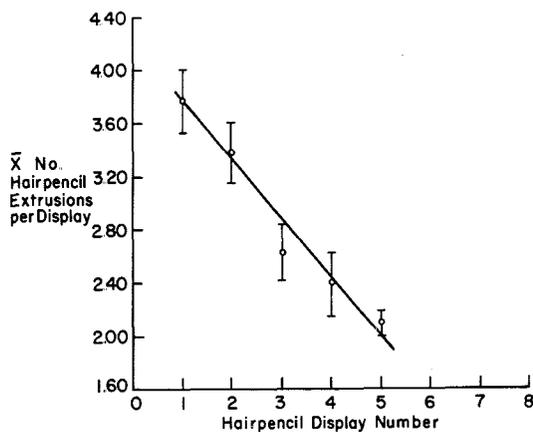


FIG. 5.—The decline of mean number of hairpencil extrusions during later displays. Brackets around means denote standard errors; solid line is weighted linear regression line $N = 22$ for display no. 1, 21 for display no. 2, 17 for display no. 3, 14 for display no. 4, and 8 for display no. 5.

did not significantly differ from each other (with 1 exception) (Fig. 9A, B). The apparent preference for the left quadrant rather than an equal left-right distribution was explained after the experiment was done when a smoke plume was generated through the apparatus. The piece of white tape concealing the tip of the pipette deflected the pheromone-laden air decidedly to the left and

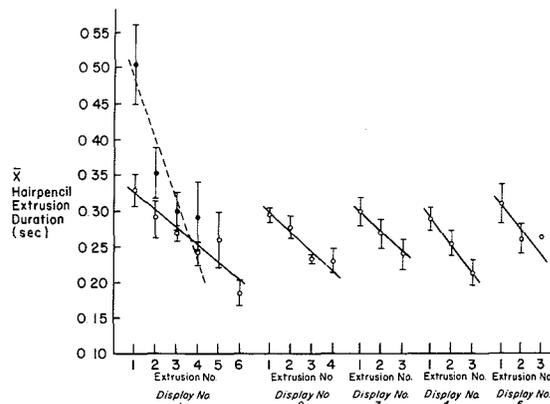


FIG. 6.—Pattern of mean durations of hairpencil extrusions within successive hairpencil displays toward: females which did not move toward and touch male's abdomen (open circles); and females which moved toward and touched male's abdomen (solid circles). Solid and broken lines are linear regression lines for the stationary and moving female groups respectively. Brackets around means denote the standard errors. Total number of observations, 231 for open circles, 62 for solid circles.

in the control situation males were spending more time in contact with the pheromone plume both downwind but also to the left of the source. The only time this changed was when the model was placed directly over the source, changing the plume's course closer to the "expected" downwind direction and altering the amount

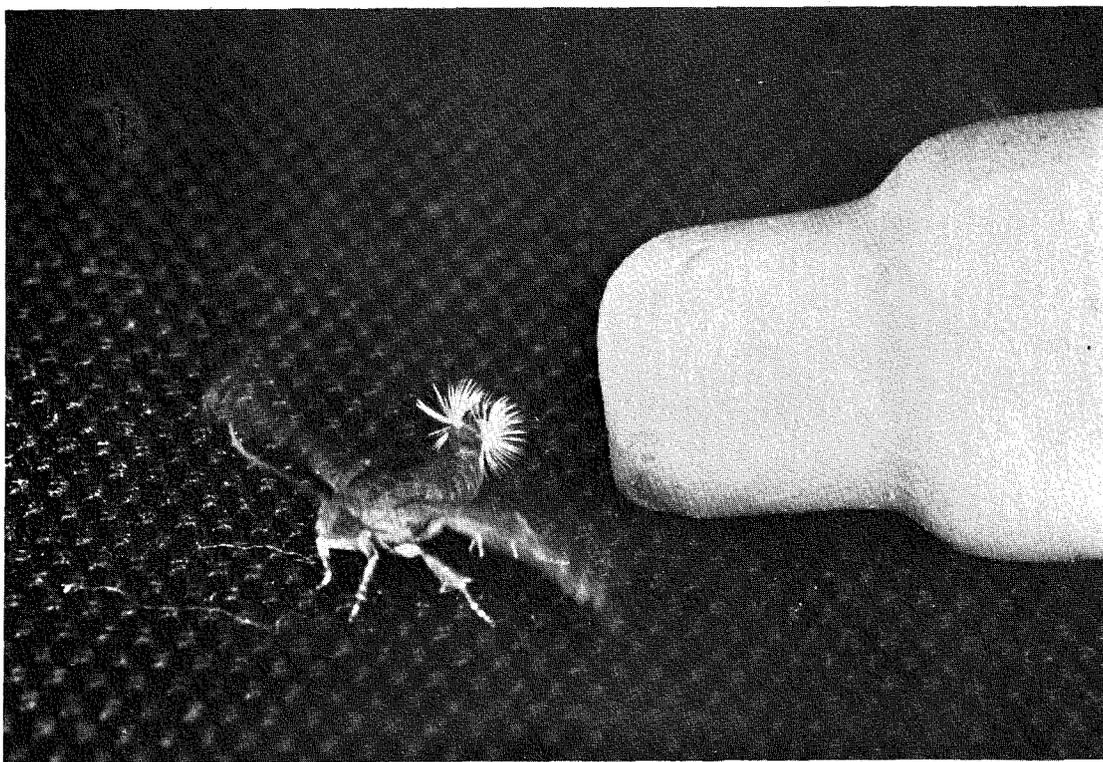


FIG. 7.—*G. molesta* male directing hairpencil display toward a rubber septum placed directly upwind of the pheromone source. Male approached on the platform from the right by wing fanning while walking and moved past the model before facing away and displaying his hairpencils.

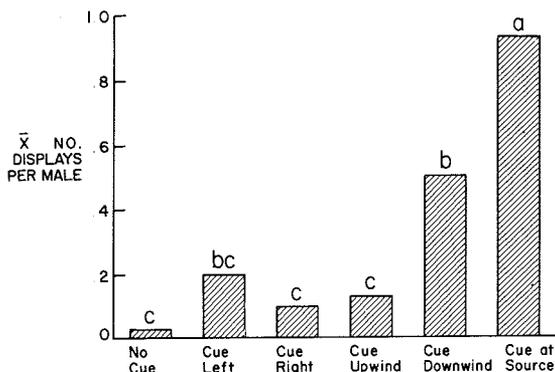


FIG. 8.—Mean number of hairpencil displays per male elicited when the visual cue (paper model) was in the position indicated. Means having no letters in common are significantly different by the LSD test ($P \leq 0.05$). For the visual cue: absent, $N = 22$; to the left, $N = 24$; to the right, $N = 26$; upwind, $N = 26$; downwind, $N = 27$; over the pheromone source, $N = 28$ males.

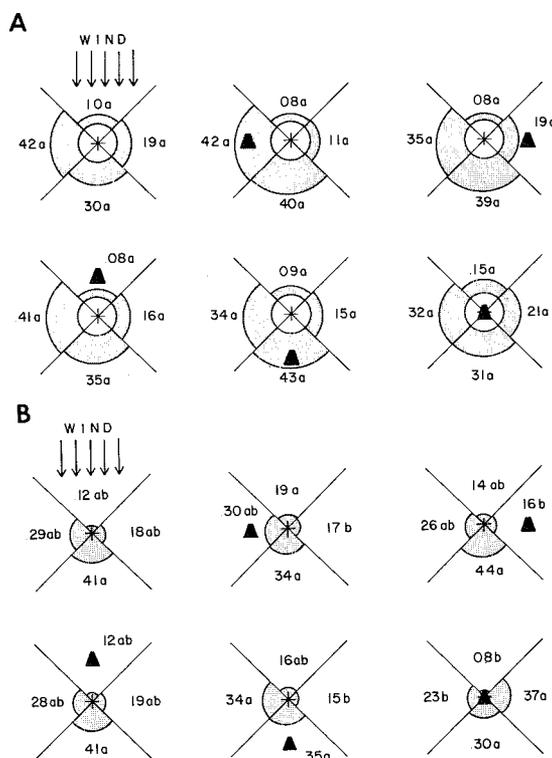


FIG. 9.—Effect of different placements of the gray paper model 1 cm from the pheromone source upon percentage of time spent by males in each of the quadrants on the observation platform. The “+” in the center of each figure is the location of the pheromone source, and the solid black figure represents the model’s position. Decimal numbers and corresponding widths of shaded areas represent the mean percentage time spent by males in each quadrant; A) away from the pheromone source ($0.5 \text{ cm} \leq X \leq 2 \text{ cm}$) and B) at the pheromone source ($X < 0.5 \text{ cm}$). For a particular quadrant, means for each of the 6 different model positions having no letters in common are significantly different by the LSD test ($P \leq 0.05$). For the model: absent, $N = 22$; to the left, $N = 24$; to the right, $N = 26$; upwind, $N = 26$; downwind, $N = 27$; over the pheromone source, $N = 28$ males.

of time spent by males close to the model (Fig. 9B) in this instance. Regardless of the absence or presence of a visual model in various positions around the source, males oriented primarily to the chemical stimulus and spent most of their time in those quadrants with a greater concentration of pheromone.

The differences in hairpencil behavior elicited by models in different positions may be explained by whether or not the visual and chemical stimuli are spatially “connected.” When they are, there is a greater chance that the display will be released, especially if the chemical and visual cues have a common origin (when the model is directly over the chemical source) (Fig. 8).

These results resemble those of Shorey and Gaston (1970) with the cabbage looper moth *Trichoplusia ni* (Hbn.). After showing that the major means of orientation in *T. ni* was chemical, they demonstrated that the frequency of copulatory attempts increased significantly when a model was placed directly over the pheromone source, and these attempts were directed toward the source more frequently when a model was present. A wide variety of 2- and 3-dimensional models was effective in evoking copulatory attempts. Similarly, Colwell et al. (1978) found that in *Pectinophora gossypiella* (Saunders) a visual model located downwind within the pheromone plume evoked male copulatory behavior more often than the chemical alone or a model located upwind from, and therefore not connected to, the pheromone stimulus. Thus hairpencil display in *G. molesta* and copulatory attempts in *T. ni* and *P. gossypiella* are more frequently evoked by, and directed toward, visual models associated with a pheromone stimulus. A major difference is that *G. molesta* is sexually active in daylight whereas *T. ni* and *P. gossypiella* are nocturnal.

Effect of Female Movement on Hairpencil Extrusion Duration

The 1st hairpencil extrusion is prolonged significantly when the rubber septum model is rotated 180° immediately after initiation of the display (Table 3). When the septum is not rotated, but the pin merely held from beneath, the extrusion duration is significantly shorter. The male apparently watches the female during his display and, if she moves, the hairpencils are held out for a longer period of time. These results explain at least in part the correlation between the number of males successful in attracting females and the duration of the 1st extrusion.

Table 3.—Effect of manual movement of the rubber septum model upon mean hairpencil extrusion durations. ***, entry in same column significantly different according to the *t*-test ($P \leq 0.001$); NS, entry in same column not significantly different according to the *t*-test ($P > 0.05$).

	Mean duration of hairpencil extrusions (\pm SD)	
	1st extrusion	2nd extrusion
Rubber septum moved	1.00 \pm 0.33 sec*** N = 19	0.25 \pm 0.13 sec NS N = 22
Rubber septum stationary	0.63 \pm 0.35 sec N = 25	0.28 \pm 0.12 sec N = 26

That visual feedback affects an apparently "fixed" behavior pattern at so late a stage in the sequence was not expected. Mere rotation of this crude model prolonged only the 1st extrusion; it is possible that more realistic models and movements toward a hairpenciling male might also prolong the 2nd.

Effect of Tactile Stimulus on Hairpenciling Males

All males for which touching with the brush was attempted during hairpencil display immediately made copulatory attempts in the brush's direction (Fig. 10B, C), whereas all males in the control group with the brush placed close to but not touching the hairpencils continued their displays and made no immediate attempts at copulation (Fig. 10A). That contact had actually occurred and not occurred in the experimental and control groups, respectively, was proven by the transfer of powder to 22 of the 23 males in the former group and lack of powder on the abdominal tips of all 21 males in the latter. The tactile stimulus given to males in other behavioral states did not release copulatory behavior; for instance, males wing fanning while walking touched with the brush either showed no change ($N = 2/19$), began walking ($N = 5/19$), or flew ($N = 12/19$). These results demonstrate experimentally that the female-delivered "touch" to the male's abdomen releases copulatory behavior in males in the hairpenciling phase of courtship.

Relative Importance of Chemical, Visual and Anemotactile Stimuli from Hairpencil Display

The most important stimulus in the hairpencil display is chemical, indicated by increased responses in all 3 behavioral categories (locomotion, upwind locomotion, touch the paper) to treatments containing hairpencil extract as opposed to solvent alone (Table 4). The hairpencil pheromone is a short-range attractant to females. Wind added to the solvent blank elicited responses significantly greater than to the solvent blank with ambient wind in all 3 behavioral categories; wind added to the hairpencil extract evoked higher levels of locomotion compared to all other treatments. Thus wind does appear to have some effect by itself in eliciting locomotion and orientation, but not so much as the hairpencil pheromone.

Blinding the females had no significant effect upon female response in any of the categories (Table 4), so vision contributed little to the orientation response of the female in this experiment. This does not eliminate vision as a cue used by females in orienting to hairpenciling males; however, its role is apparently minor compared to the olfactory and anemo-tactile modalities.

Measurement of Wind Velocities Generated During Hairpencil Display

Males hairpenciling from 1–2 cm directly upwind of the gray paper model (Fig. 11) generated wind from their vibrating wings generally at 2 basic velocities. During extrusion the mean maximum velocity was ca. 45 cm/sec (~1 mph), and during retraction it increased to 90 cm/sec (~2 mph). Changes in the apparent angle of wing vibration appears to accompany these wind velocity changes. If the female were 1 cm away, the pheromone could be puffed from the hairpencils to the fe-

male in ca. 1/45 sec. Also, since it would always originate at the male, the wind would impart a directionality to the signal and allow the female to use pheromone-mediated positive anemotaxis to locate him.

Measurement of Female Preference for Males with Intact Hairpencils

Females are attracted only to males able to extrude their hairpencils during display. Males with glued abdomens evoked no female movement in 169 displays (Table 5). No aspect of male courtship behavior other than hairpencil extrusion differed from "normal" courtship. The number of attempts at extrusion could be observed during each display by watching changes in the angle of wing vibration which accompany changes in wind velocity. Sham-operated males were attractive to the same females which rejected hairpencil-less males immediately following the latter males' unsuccessful courtship attempts (Table 5). That the dried glue was not the cause of diminished female response to operated males was proven when females responded no differently to sham-operated males bearing dried glue on their thoraces than to the other sham-operated males (Table 5). Females thus have a clear "preference" for males able to extrude their hairpencils during display, and the basis of the female response (or lack of one), as demonstrated earlier, is largely chemical. As observed many times during the course of this study, males having naturally deformed, reduced, or incompletely extrusible hairpencils appeared less able to attract, and less likely to copulate with, calling females.

Discussion

Grapholitha molesta Courtship Behavior

G. molesta courtship behavior is unique among the Lepidoptera studied thus far in that it includes a display of hairpencil organs by the male whose chemical stimuli attract the female over a range of 1–2 cm after he already has been attracted to the female by her sex pheromone. Males of the lesser wax moth, *Achroia grisella* (F.), attract females over longer range by a 2-component pheromone emitted from wing glands and the sound of vibrating wings (Dahm et al. 1971), and thus the *A. grisella* male pheromone acts as a long-range attractant. In the greater wax moth, *Galleria mellonella* (L.), the male-emitted wing-gland pheromone evokes similar behavior in conspecific females (Röller et al. 1968, Leyrer and Monroe 1973, Finn and Payne 1977). In the Indian meal moth *Plodia interpunctella* (Hbn.) (Grant and Brady 1975), and *Vitula edmandsae* (Packard) (Grant 1976a), a combination of male hairpencil pheromone and tactile cues causes the female to turn 180° to face a male approaching from the rear or side. These appear to be the only other occurrences of pheromone-evoked female movement to males in the Lepidoptera. Apart from *P. interpunctella*, *V. edmandsae*, and *G. molesta*, all other lepidopteran male courtship pheromones elicit in females "acceptance" by quiescence (Tinbergen 1942, Brower et al. 1965, Birch 1970, Pliske and Eisner 1969, Grant and Brady 1975) and/or abdominal extension (Tinbergen 1942, Grant and Brady 1975, Rutowski 1977). In some species, the hairpencils, although ac-

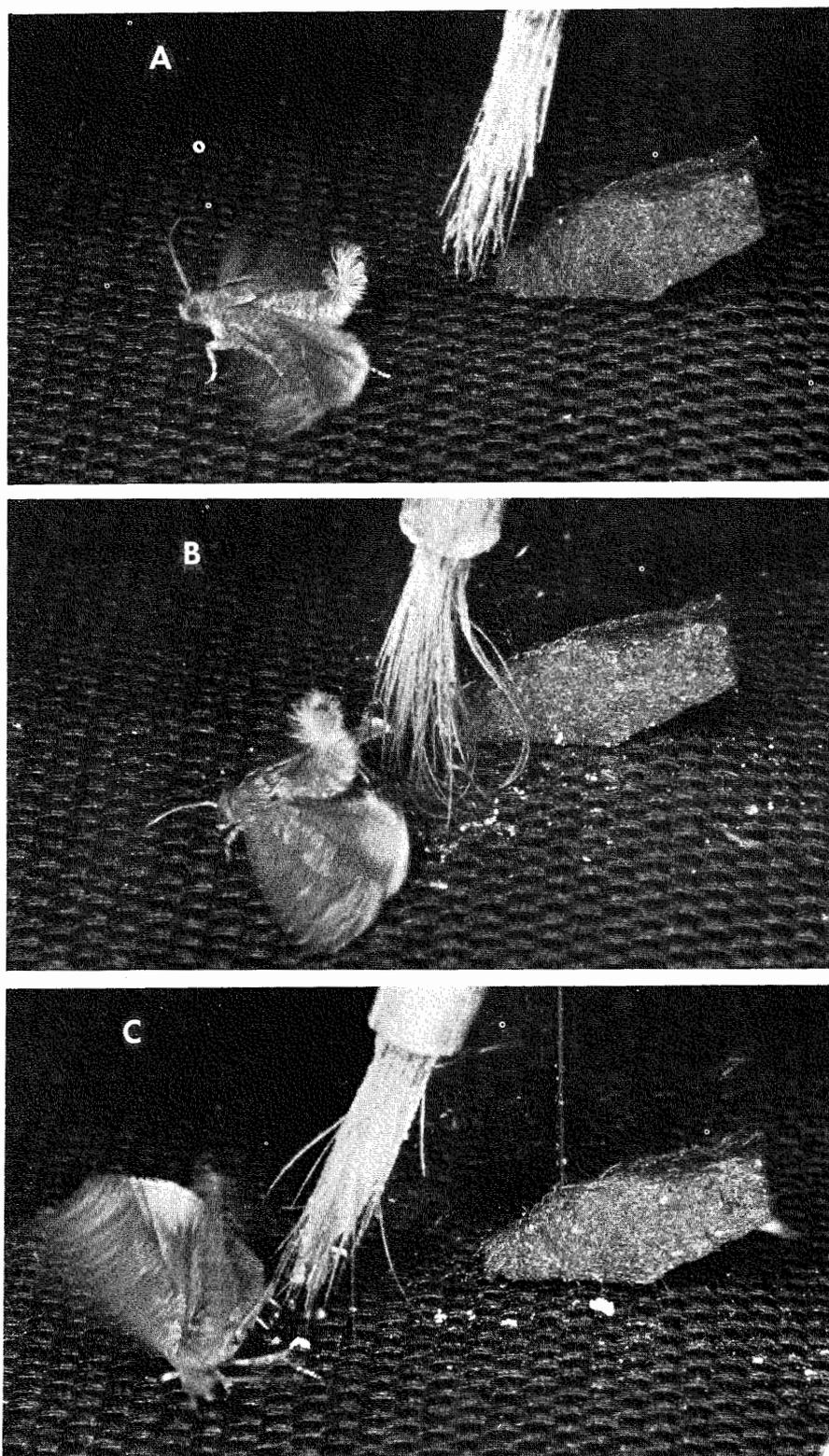


FIG. 10.—Effect of tactile stimulus applied to male abdomen during hairpencil display: A) brush dusted with red fluorescent powder and placed near, but not touching male; B) contact made with abdomen (note initial phase of copulatory attempt which includes a hyperextension of the abdomen; C) beginning of second phase of copulatory attempt, in which male turns quickly around and extends claspers.

tively displayed, have little or no behavioral effect upon females, and when removed have no effect upon male success rate in mating (Gothilf and Shorey 1976, Pliske 1975, Grant and Brady 1975). In the case of the monarch butterfly, *Danaus plexipus* (L.), whose hairpencil

organs are somewhat reduced in size and apparently lack sufficient pheromone to influence female behavior, males rely upon tactile stimuli, aerially grabbing the female to force acquiescence when the pair hits the ground (Pliske 1975). Male queen butterflies, *D. gillipus* (Cramer),

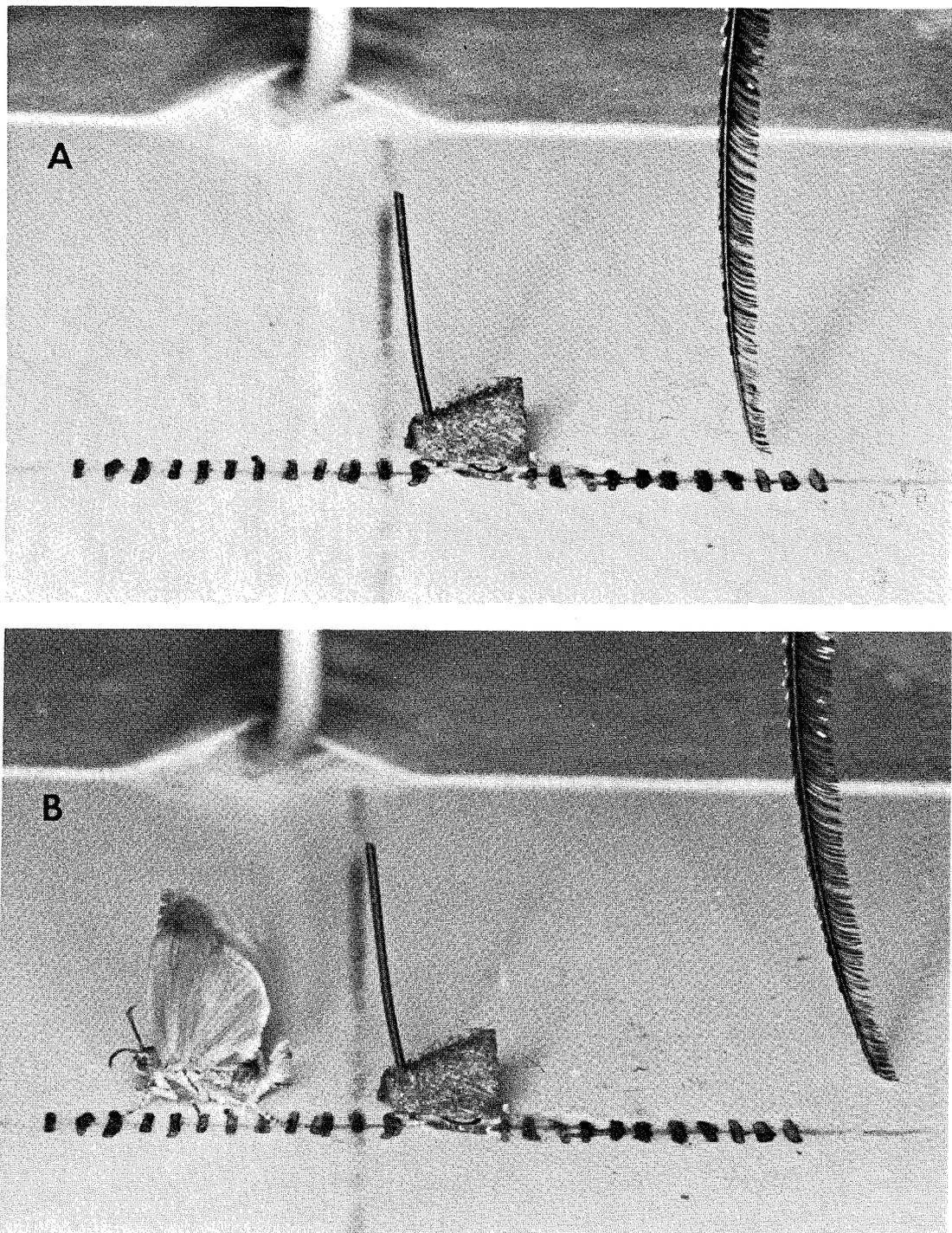


FIG. 11.—The feather anemometer: A) in resting position with an ambient wind flow from left to right of 0.7 m/sec; B) deflected to the right by wind generated during hairpencil display by the male's vibrating wings.

Table 4.—Responses of blinded and sham-operated calling females to filter paper impregnated with male hairpencil extract or solvent alone and held 1 cm upwind on a horizontal observation platform. Mean ambient wind velocity was 33.3 cm/sec (± 17.29) and wind was also added by blowing filtered air at a mean velocity of 76.3 cm/sec (± 12.58) over the filter paper and onto the female. Locomotion involved walking in any direction, upwind locomotion was defined as any walking movement which included at least some direct upwind motion, and touching the paper included those females which walked upwind and touched or walked onto the filter paper. Percentages in any column having no letters in common are significantly different according to a $\chi^2 2 \times 2$ test of independence with Yates' correction ($P \leq 0.05$).

				No ♀ tested	Locomotion	Upwind locomotion	Touch paper
Solvent blank	No wind added	Not blinded		86	5 (5.8%) d	3 (3.5%)c	2 (2.3%) c
		Blinded		92	4 (4.3%) d	2 (2.2%) c	2 (2.2%) c
	Wind added	Not blinded		86	22 (25.6%) c	17 (19.8%) b	8 (9.3%) bc
		Blinded		92	27 (29.3%) bc	18 (19.6%) b	13 (14.1%) b
Hairpencil extract	No wind added	Not blinded		92	40 (43.5%) b	34 (37.0%) a	29 (31.5%) a
		Blinded		92	37 (40.2%) bc	33 (35.9%) a	27 (29.3%) a
	Wind added	Not blinded		92	59 (64.1%) a	44 (47.8%) a	33 (35.9%) a
		Blinded		92	55 (59.8%) a	44 (47.8%) a	32 (34.8%) a

Table 5.—Responses of females presented first with males unable to extrude their hairpencils followed immediately by sham-operated males with apparently normal hairpencil extrusions. A second control group was comprised of sham-operated males with glue applied to their thoraces which did not hinder hairpencil extrusion; these males were exposed to naïve females. Entries in same column having no letters in common are significantly different according to a $\chi^2 2 \times 2$ test of independence with Yates' correction ($P \leq 0.001$).

	No. ♀ tested	No ♀ attracted to and touching ♂ abdomen	No. ♀ in which at least locomotion was evoked	Total no. displays
Males with glued hairpencils	29	0 (0%) b	0 (0%) b	137
Sham-operated males (same females as used above)	29	20 (69%) a	26 (90%) a	61
Sham-operated males with glue on thorax (naïve females)	25	16 (64%) a	22 (88%) a	129

aerially induce female quiescence by means of hairpencil pheromones alone from their well-developed organs (Brower et al. 1965, Pliske and Eisner 1969); hairpencil removal results in an inability to "seduce" females and a decrease in mating success (Pliske and Eisner 1969).

In *G. molesta*, correctly performed courtship behavior is necessary for the highest frequency of mating success. If a male touches a female as he walks past while wing fanning during the earliest stages of courtship, she walks or flies away. Likewise, an unattractive hairpencil male attempting copulation is not likely to succeed (Fig. 4B); again, the female either walks or flies away.

G. molesta courtship involves a complex series of behavioral responses and corresponding signals sent in at least 3 sensory modalities over ca. 1.5 sec; but basically it is a "chemical dialogue" between a calling female and an answering hairpencil male. That males use a chemical for rapid communication during the 1 or 2 sec in which courtship proceeds, at first appears unusual. Among the available modalities, chemicals could be considered the least efficient for rapid communication because their transmission depends upon diffusion or, at best, ambient wind velocity and direction; the last 2 are

subject to high variability. *G. molesta* males overcome these limitations by supplying their own wind, using wing vibration to propel the chemical; the signal will reach a female 1 cm away in 1/45 sec or less. In addition, the self-generated wind gives the chemical signal directionality regardless of a male's position with respect to ambient wind direction, and may enable the female to use upwind anemotaxis to locate the male. During hairpencil display, the male is usually upwind or sidewind of the female and always faces away from her, generating wind posteriorly; the series of extrusions and retractions must continue under reduced, or perhaps in the absence of, female pheromone stimulation. Therefore, after its initial release, each display appears to exhibit "momentum" (Lorenz 1950), the male obtaining renewed pheromone stimulation only when he faces the female between displays.

Visual stimulation, however, is present at all times. Although the male courtship sequence is a fairly rigid fixed action pattern, visual feedback in the form of female movement toward the male can alter the form of the hairpencil display. The hairpencil male apparently watches the effect of his display upon the female;

if movement results, the hairpencils are held out longer. If the female is slightly out of alignment when she reaches the male, he will sometimes move his abdomen toward her to make contact with her head, although this may be due to tactile as well as visual stimuli. In addition, the hairpencil display is released by the visual stimulus of a calling female's body combined with a precise blend of female-emitted pheromone chemicals (Baker and Cardé, unpubl., Cardé et al. 1979).

It is not clear why males pulse their chemical and anemo-tactile signals. It may be that males not successful in attracting the female during the 1st extrusion retract their hairpencils in order to "recharge" them. Hairpencils of successful 1st extrusion males might have a reduced need to be recharged and so are extruded longer. Possibly a pulsed rather than continuous signal contains more information, but it remains to be demonstrated that such an amplitude-modulated signal would cause a greater female response.

The courtship behaviors of *V. edmandsae* (Grant 1976a), *P. interpunctella* and the almond moth, *Cadra cautella* (Walker) (Grant and Brady 1975), include eversion of male hairpencils from the forewing costal margins plus apparent male-female exchange of visual and tactile stimuli, and are therefore similar in complexity to *G. molesta* courtship. Unlike those of *V. edmandsae* and *P. interpunctella*, *C. cautella* hairpencils have little or no effect upon mating success. Males do have, associated with the claspers, an auxiliary set of brush organs which are everted during an "unusually prolonged" copulatory thrust (Grant and Brady 1975) and may influence female acceptance. These abdominal organs appear to be similar to those of *G. molesta* and the other *Graepholitha* species investigated by Heinrich (1926).

Possible Role of Sexual Selection in the Evolution of Male Hairpencils

An outstanding problem in the evolution of courtship behavior in Lepidoptera is the combination of behaviors not easily explained by natural selection: a high degree of female coyness accompanied by correspondingly elaborate male hairpencil behavior. For reproductive isolation, a species-specific chemical signal from the male would appear to be taken care of nicely by a small quantity of a specific chemical blend, as it has for female-emitted pheromones over a much longer distance. Yet the trend in the Noctuidae is for the structures of chemicals released in copious amounts to be most similar among closely related species and to diverge among more distant relatives (Birch 1974). Similarly, Manning (1966) found a direct correlation between phylogeny and courtship behavior in *Drosophila*, and concluded that the relationship should be an inverse one for species-specific behavior. The chemical specificity of the danaines also does not appear very precise, even though there are large quantities of compounds present; however, this could be due to an emphasis upon visual cues typical of butterfly courtship.

Besides reproductive isolation (Grant et al 1975, Grant 1976b), other evolutionary hypotheses have included hairpencils' possible importance as release surfaces for primer pheromones that stimulate oögenesis, deterrents to other males, and long-range sex attractants

presumably before this function was taken over by female pheromone glands (Birch 1974). Each of these ideas has some merit, and yet, as Birch (1974) states, "The question remains as to how the male brush-organs, hair-pencils and coremata originally evolved." It may be that to answer this question, one need look no further than the primary role they play in eliciting female acceptance, because in *G. molesta* courtship and that of other lepidopteran species, it is the female who ultimately accepts or rejects a displaying conspecific male through her behavioral response. We suggest that *G. molesta* hairpencils and behavior as well as those of some other male Lepidoptera may have arisen primarily as a result of "female choice" sexual selection (Fisher 1958).

In his theory of sexual selection, Darwin (1898, p. 335) wrote of insects: "When we see many males pursuing the same female, we can hardly believe that the pairing is left to blind chance—that the female exerts no choice, and is not influenced by the gorgeous colours or other ornaments with which the male is decorated." Richards (1927, p. 348) hypothesized that female "coyness" or hesitation to mate was the reason insect displays evolved, yet did not appear to favor a sexual selective mechanism to explain how selection for these displays would proceed. Indeed, he concludes, "... Although the displays, considered as a whole, have a survival value, yet it is difficult to see any use in the specific differences in secondary sexual structures of behaviour. It is not at all evident how selection could establish, in a population, a character whose only effect would be to isolate individuals possessing it from those who did not."

Fisher (1958) broadened the distinction made by Darwin (1898) between the type of sexual selection based on male fighting or other direct interactions in which the "winning" male mates with the receptive female, and the type in which a male display directed toward a female causes her to "choose" him over the other males for copulation. In the female choice type, the preferred trait(s) may be important solely for attracting females, thereby increasing mating success, apparently like the plumage of male birds of paradise (Mayr 1972). In Fisher's model, a female preference character for a male character is initially favored by natural selection and at a low frequency in the population. Female preference behavior rapidly becomes genetically associated with the preferred male character because discriminating females are more likely to choose only males having the preferred character. Such females will pass on more of their genes because their sons will more likely be accepted for mating by discriminating females in the next generation. Before long both the male character and female preference behavior will develop beyond the level they might reach through natural selection. O'Donald (1962, 1967) formulated mathematical models substantiating Fisher's hypothesis; in his models the "runaway" tendency of sexual selection eventually is opposed and slowed by natural selection.

There is evidence in *Drosophila* of rapid sexual selection for differences in male courtship behaviors due to competitive mating under laboratory conditions, and these disappear when pairs are coupled in separate bottles, removing the competitive aspects of mating (Ewing

1961). Ehrman (1972) demonstrated frequency-dependent sexual selection by females who "choose" rare males for mating under laboratory conditions. Sexual selection was suggested by Manning (1966) to be linked to the evolution of male courtship behavior in *Drosophila*, possibly through selection for male behaviors correlated with vigor or fitness; female coyness would allow time to assess the male's stimuli before accepting or rejecting him. Grant (1976b) viewed reproductive isolation as a possible reason for the evolution of female coyness and corresponding male courtship behavior in the moth *P. interpunctella* due to the species-isolating effects of its courtship behavior and that of another closely related moth, *C. cautella* (Grant et al. 1975).

Two fundamental conditions must be met for sexual selection to occur. First, there must be a sexual preference in at least one sex and, second, there must be "bionomic conditions in which such preference shall confer a reproductive advantage" (Fisher 1958). The latter conditions—including protandry, male-female sex ratio favoring males, and a broader male than female daily sexual activity period—favor polygyny and, combined with sexual preference, result in the mating of some males more frequently with more females at the reproductive expense of other males. For *G. molesta* (this paper), *D. gillipus* (Pliske and Eisner 1975), a noctuid moth (Birch 1970), *P. interpunctella* (Grant and Brady 1975), and *V. edmandsae* (Grant 1976a), sexual preference was demonstrated when females mated preferentially with males possessing intact hairpencils. The major hairpencil stimulus was in all cases apparently chemical. Bionomic situations favorable for sexual selection commonly occur in the Lepidoptera. For example, *G. molesta* males confined in cages with an excess of virgin females are capable of mating an avg of 1.2 times/day during their 1st week as an adult. Spermatophore counts of wild females indicated that the majority (79% over the 3-yr study) had mated only once and most of the rest only twice; of caged females supplied with an equal number of males, 60% mated once, 20% twice, and 10% 3 times over a 21-day period (Dustan 1964). Thus males can mate at least once per evening whereas most females will mate only once during their lifetime. In addition, our field observations indicate that a calling female or a synthetic pheromone dispenser may be visited by multitudes of males either simultaneously or within a few seconds of each other.

The evolution of hairpencil behavior in the Oriental fruit moth might have proceeded in the following sequence. First, a male character arises for which the corresponding female's preference is favored by natural selection. The female preference behavior might entail hesitating slightly longer than normal during courtship until an assessment of the male character can be made. The initial frequency of female preference alleles in the population could be extremely low. The preferred male trait might indicate: (1) male "vigor" or quality (through behavioral response or quantity of chemical odor on body); (2) gender or species (e.g., male-specific odor or species-specific behavior or odor). Males possessing an odor developed as a deterrent to predators also could be preferred by females. If the species was polygynous, promoting increased mating frequency for preferred

males, then linkage of female preference with the male trait could occur; rapid directional selection would predominate until a stable equilibrium was reached. The rate of selection would depend upon the severity of male exclusion, the initial allelic frequencies, whether the trait was dominant or recessive (recessives are favored), and the resistance offered by natural selection (O'Donald 1967). Before the process would slow down, however, the directional selection could result in an exaggerated scent-dissemination mechanism or courtship behavior. In addition, the reciprocal directional selection for female coyness behavior could result in a higher degree of hesitancy to mate.

The present *G. molesta* hairpencil display may represent a type of clasper extension "ritualized" through the process of sexual selection. It may have originated from a genital odor emitted coincidentally during a prolonged copulatory attempt involving clasper extension. Through sexual selection, scent-disseminating structures associated with the claspers could have become highly developed and the corresponding clasper extension prolonged or even repeated several times in succession. The concurrent directional selection for female preference behavior may have resulted in the coyness currently exhibited by *G. molesta* females: they walk or fly from males who either touch them before hairpencil or attempt copulation after a nonattractive display. Sometimes a male may display several times before the female moves and touches his abdomen.

For other polygynous Lepidoptera, female preference could conceivably develop for any of a wide variety of male traits. The diverse group of hairpencil structures observed today may be a result of the possibly cavalier way a male trait could be favored and then exaggerated by rapid sexual selection. Certain groups having been exposed to a higher incidence of female preference initiators such as incomplete reproductive isolation by female pheromone, may be those most likely to have evolved functional hairpencils and female "coyness." Sexual selection involving direct male-male fights and competition near a receptive female (Darwin 1898, Fisher 1958), considered natural selection by many authors (Mayr 1972), may be another mechanism favoring the evolution of hairpencils in other species. In Hawaiian *Drosophila*, Spieth (1968) found evidence of inter-male aggression near their leks and sexual dimorphism appeared to be greatest in lekking species. However, observation of *G. molesta* indicates male displays actually attract, rather than deter, other males, the converse of what might be expected if competition or fighting were occurring. Such attraction and homocourtship also occurs in *V. edmandsae* (Grant 1976a).

Cross-attraction with *Grapholitha prunivora* (Walsh), the lesser appleworm, does sometimes occur at a level of less than 5%, and male *G. prunivora* courtship behavior also includes a hairpencil display. However, other steps in its courtship sequence differ significantly from that of *G. molesta* (Baker and Cardé, unpublished). If sexual selection caused the development of hairpencil behavior in these species, the initially preferred male trait might have been a species-specific male odor or behavior, although perhaps not between these 2 species; they have been sympatric for less than a century. Further

studies of male courtship and female sex pheromones may define the relative importance of sexual and natural selection in the evolution of chemical communication in the genus *Grapholitha*.

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