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Variations in male oriental fruit moth courtship patterns due to male competition

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Summary. In a laboratory flight tunnel, the first oriental fruit moth males to arrive near females had the highest rate of mating success and performed the standard sequence of behaviors including a hairpencil display that attracted females. Late arrivals performed atypical courtship patterns that enabled them to sneak copulations or disrupt first males' courtships to succeed in mating occasionally.

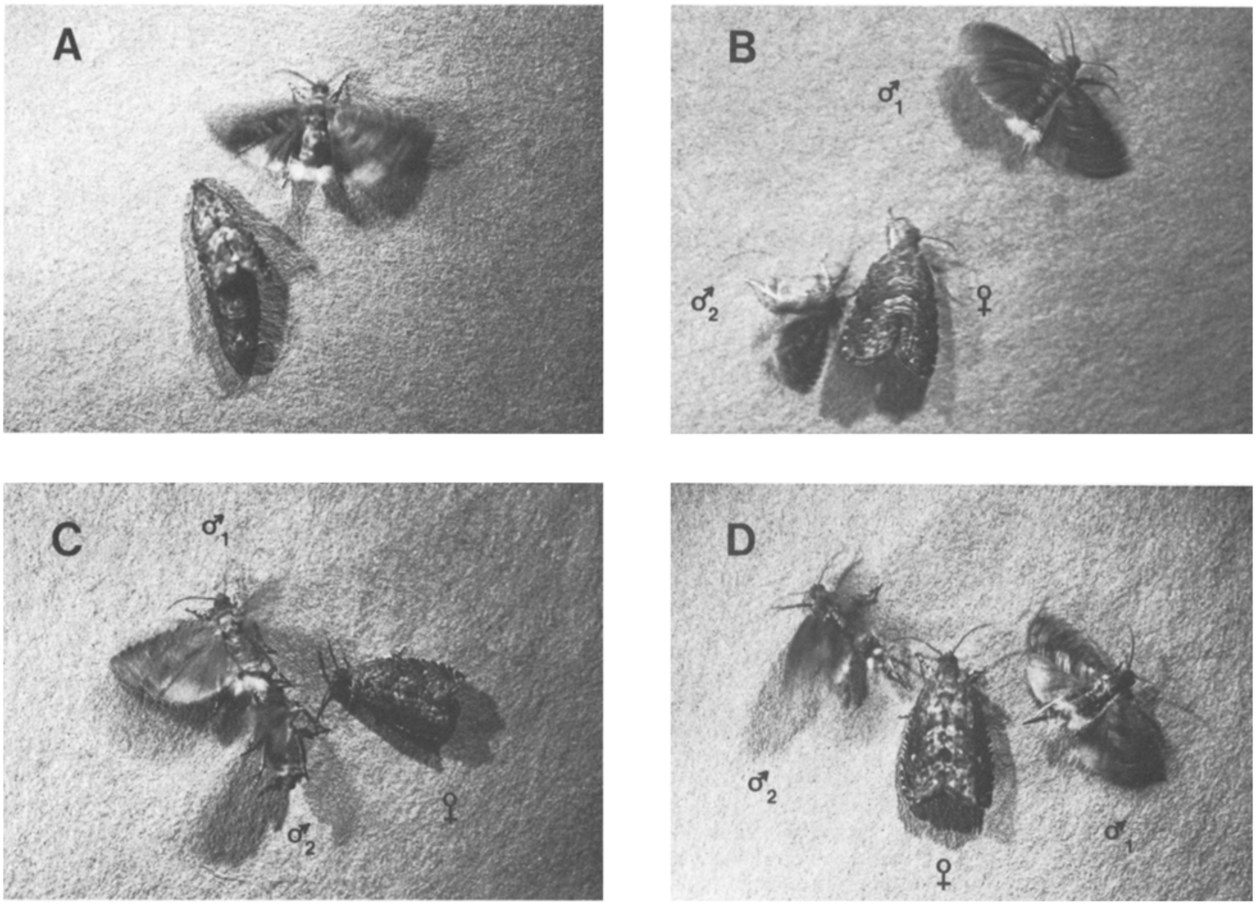
In the absence of other males, male oriental fruit moths, *Grapholitha molesta* (Busck), attracted to the vicinity of a calling female by her sex pheromone perform a stereotyped sequence of behaviors culminating in a rhythmic extrusion and retraction of the hairpencil organs toward the female[2]. Females are attracted to the end of the male's abdomen by a blend of at least 2 chemicals identified from hairpencil extracts, *trans*-ethyl cinnamate and methyl-2 epijasmonate[3,4]. Performing the hairpencil display is a prerequisite to successful mating by solitary courting males[2]; only a male that attracts a female to and receives her touch upon his abdomen will have a chance of mating. In observing males near synthetic sex pheromone sources and during courtship with females, it became apparent that when many males were present, some moved toward other's displays and touched their hairpencils in a manner similar to females. This usually evoked a copulatory attempt by a displaying male and raised the question of why males would engage in this type of behavior. To study the matter in more detail, encounters were set up near calling females that would result in male-male interactions and where the mating success of males performing such variant courtship behaviors could be measured. This paper reports these results, which show that the typical courtship pattern exhibited by solitary males is not as successful when used in the presence of competing males, who use other tactics to mate under competitive conditions.

G. molesta were reared on small green apples and segregated by sex as pupae according to the number of abdominal segments. Adult males were held in separate chambers from females, and both were on a 16:8 light:dark photoperiod regime. At the time of peak responsiveness to sex pheromone by males and pheromone gland extrusion by females, beginning about 2 h before lights-off[5], an untethered female exhibiting calling behavior was transferred from a vial onto a 0.15×0.15×0.15 m low turbulence, sheet-metal platform (hollow, and all surfaces parallel to the windline) in wind of 0.5 m/sec in a 3.4×1.0×0.9 m Plexiglas® wind tunnel[6]. After the female began calling again on the platform, a group of 4 males was held in the pheromone plume about 1.5 m downwind of the female so that 2 or more of them took flight upwind toward the female. All courtship and competitive interactions were videotaped from directly above the platform by a Sony RSC 1050 rotary shutter camera and analyzed frame-by-frame on a Sony SVM 1010 motion analyzer.

The presence of late-arriving competitors severely reduced the mating success of 1st-arrivals. 'Competition' was defined as occurring when a 2nd male landed on the platform before a copulatory attempt by the 1st-arriving male[2] (a curling of the abdomen toward the female's abdomen, concurrent with head-push by the male, as in fig. B). 'Arrival' was the moment a male landed and began wing-fanning while walking on the platform. With no late-arriving competitors, 1st-arrivals experienced a 72% level of mating success (55/76) whereas in the presence of competitors their success was lowered to 14% (3/22) (χ^2 2×2-test of independence; $p < 0.05$). 1st arrivals that never successfully displayed at females (N = 10), usually because they touched them prematurely during their approach, were not considered to have courted females and were not included in these success percentages. There were only 22 instances of competition despite the nearly simultaneous take-off and upwind flight of several males from the release cage because 1st-arrivals provided a very small time period during which competitors could arrive before copulation had already occurred. 1st-arrivals experiencing no competition began their displays an average of 2.04 sec (± 1.50 SD) after landing, and attempted copulation 3.62 sec (± 2.24 SD) after landing, completing the coupling in 4.54 sec. The next males did not arrive until 8.62 sec (± 10.99 SD) after the 1st males and so it is clear that those males arriving first and accomplishing their courtship displays quickly were at a mating advantage. Late-arrivals only succeeded in mating 21% of the time (12/56) but their chances were better (41%; 11/27) if they arrived in time to compete with the 1st-arrivals, i.e., before a 1st male had made a copulatory attempt.

Males arriving after 1st males were already coupled with females nevertheless often displayed at the mating pair (fig. A). In 9 out of the 29 times this occurred (31%), the female was attracted at least part way to the displaying male, forcing the male to which she was coupled to move with her. In 2 of these 9 cases the female became disengaged from her mate and touched the 2nd male's abdomen, and one of these females ended up coupling with the 2nd male. Therefore the 'strategy' of giving the hairpencil display to already coupled females occasionally may result in a successful mating (under these conditions 1/29 times, or 3%), and presumably might be worthwhile considering the time and energy needed to locate and court a new female. Dislodging the 1st male from the female with the display or even a copulatory attempt might not be as difficult as it first would seem, because the 1st few sec of linkage sometimes are insecure. Additionally, in the 1st few sec of coupling some solitary males apparently failed to link solidly with the female. They disengaged themselves from the female and repeated the typical courtship sequence, finally attracting her again to achieve a more secure coupling.

Late arrivals landing before the 1st male had made a copulatory attempt had much better odds of mating (11/27, or 41%) than those courting coupled pairs. In their competitive interactions with 1st arrivals, these males employed a variety of behaviors, all of which were sharp departures from the typical courtship sequence. One variation was a copulatory attempt without the customary hairpencil display. Late-arriving males would overtake and intercept females being attracted to the 1st-arrival's display, and from the atypical side position thrust their abdomen toward the (moving) female before she could touch the displaying male's hairpencils (fig. B). Such males were successful 46% of the time (6/13) in gaining a copulation, and this is the only situation observed thus far in which *G. molesta* males mate successfully without first displaying. Under these conditions 1st males' successes were reduced to 23% (3/13).



A Late-arriving *G. molesta* male displaying his hairpencils (white tufts at end of abdomen) at pair in copula. *B* Late-arriving male (♂ 2), without displaying, attempting copulation with a female who was being attracted toward the 1st-arriving male's display(♂ 1). *C* Late-arriving male (♂ 2) about to touch the abdomen of a hairpencil-displaying 1st-arrival (♂ 1), eliciting a misdirected copulatory attempt by ♂ 1 toward ♂ 2. *D* 2 males displaying simultaneously toward a female, who eventually touches the abdomen of ♂ 1.

It is possible that the courtship pheromone released by the 1st male both makes the female receptive to such an aberrant copulatory attempt, as well as triggering the 2nd male to attempt copulation without first displaying. The 2nd male appeared to be 'sneaking' a copulation under the cover of the 1st male's chemical emission in a manner similar to 'silent' male crickets who intercept females walking toward singing males[7].

A 2nd variation was the attraction of late-arriving males to 1st-arrivals' hairpencil displays (fig. C). The resulting touch delivered to the displayers' abdomens caused them to attempt copulation with the late male, and reduced 1st-arrivals' mating success to 0% (0/12). 4 out of 12 (33%) late males who touched 1st males' abdomens, however, mated successfully by then attracting females to their own hairpencil displays. Therefore male-male attraction appears to allow late arrivals to break up the normal courtship sequence by triggering a misdirected copulatory attempt by the 1st male. Interestingly, under noncompetitive conditions the touch is only usually delivered by females, and this performance by males may be a form of female-mimicking behavior[8].

A 3rd tactic also occurred in competitive interactions between males. In 2 instances the 2nd male began displaying at the female simultaneously with the 1st male (fig. D), and the female both times 'chose' the 2nd male by touching his hairpencils. One of these 2 instances resulted in successful mating by the 2nd male. Of course, the female choice sexual selection possibly implicated here need not depend on simultaneous displays by 2 or more males. Indeed, this study illustrates the low probability of 2 males displaying simultaneously even under ideal conditions, due to the differences in upwind flight speeds among simultaneously released males and the speed with which courtship is completed by 1st-arrivals. Presumably similar factors in the field would make simultaneous displays by 2 or more males a rare event. Comparisons among males' displays might be made sequentially by females over a short period of time, however, and such female choice sexual selection could have been a primary force behind the evolution of male scent-disseminating behavior in this and other species of Lepidoptera. Nevertheless, these observations indicate that when a 2nd male arrives and begins displaying alongside a 1st male, females will sometimes choose the 2nd male, and hence this simultaneous display strategy by late-arrivals could result in reproductive success through a side-by-side comparison by females in addition to a sequential one involving several solitary courting males.

Some males performed more than one alternative courtship pattern in quick succession, and so it is unlikely that any of these behaviors are genetically fixed exclusively in some males as in some cricket species[9]. Rather, each male's behavior appeared to be quite plastic, similar to courtship in scorpionflies[10], and depended on the presence or absence of competitors and the stimuli provided by them at a given instant. These stimuli, as yet, remain unknown.

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4-Aminopyridine and fiber potentials in rat and human hippocampal slices

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Summary. Compound fiber action potentials of stratum radiatum afferents in slices from human and rat hippocampus are shown to be prolonged by 4-aminopyridine (4-AP). This action can explain the enormous increase in synaptic transmitter release caused by 4-AP.

4-Aminopyridine (4-AP) and some related compounds increase synaptic transmitter release in the peripheral and central nervous systems[1-5] presumably through a block of potassium channels leading to a prolongation of action potentials in unmyelinated nerve fibers and terminals[6,7]. Such an action may be beneficial in demyelinating diseases and some other pathological conditions, and some promising clinical tests have indeed been performed[4,6]. In hippocampal slices of the rat, we have recently shown that 4-AP increases excitarory and inhibitory transmitter release without changing the electrical characteristics of the postsynaptic membrane[1,8]. Similar results were obtained in ganglia[9] olfactory cortex[10] and the dentate area of the hippocampus[11]. At a concentration of 10^{-5} M, 4-AP caused spontaneous, sometimes seizure-like discharges and an increase of synaptic potentials (spontaneous and evoked) without altering the time course of intracellulary measured action potentials and extracellulary recorded population spikes of CA 1 pyramidal cells. The epileptiform activity is not due to a GABA antagonism[1,12]. The input volley, which is a summed action potential of afferent fibers activated by electrical stimulation, was also unchanged. If spike broadening occurred in the terminal regions of the afferent fibers it should be detected as a prolongation of the input volley. Since this potential is small, and often merges with the much larger synaptic potential under normal conditions we have now investigated the action of 4-AP in conditions where synaptic transmission was blocked. In this way, much larger and purer fiber potentials could be studied without contamination by synaptic effects.

Our methods for preparing and maintaining brain slices have been described previously[1,13]. 450-μm-thick slices were cut from the hippocampi of 11 rats and a human hippocampus, removed by hippocampectomy for treatment of drug-resistant limbic epilepsy[14]. The slices lay on a nylon mesh, in a triple version of our perfusion chamber, were

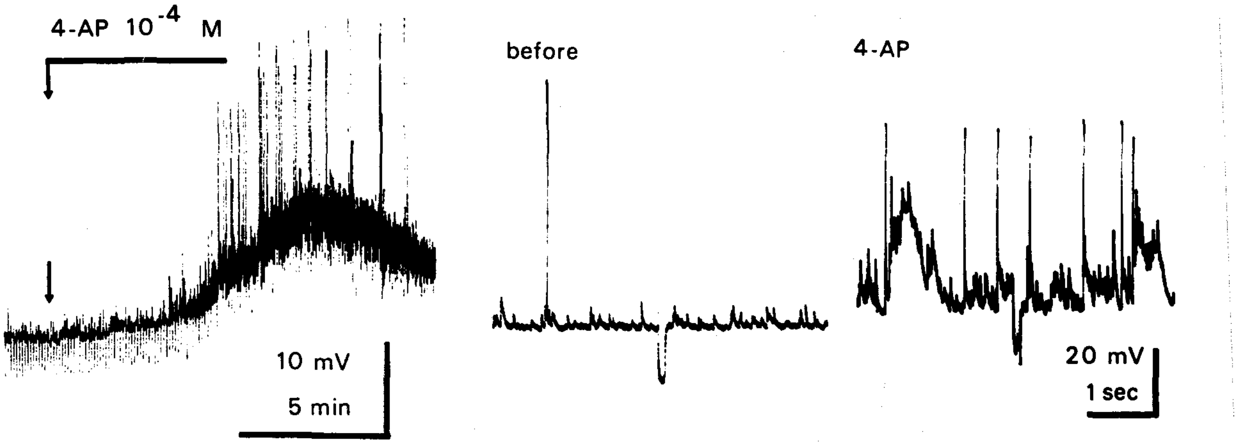

Figure 1. Action of 4-aminopyridine (4-AP) on a CA 1 pyramidal cell in the hippocampus, recorded intracellulary with a KCl-filled microelectrode (all synaptic potentials are depolarizing). 4-AP causes a depolarization of the membrane potential (left trace, which is from a pen recorder with low frequency response), an increase in synaptic and action potentials (thickening of left trace) and depolarizing shifts (slow upward deflections in left and right trace). Downward (negative) deflections are from hyperpolarizing current injection.