The acceptance response of *Ephestia cautella* (Walker) (Lepidoptera: Phycitidae) females, obtained in the absence of courting males

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Summary. Ephestia cautella females will respond to a jet of air with an acceptance posture, and will respond more frequently when the air carries volatiles, probably male sex pheromones, from the male wings.

Male phycitid moths of the genera *Ephestia* Gn. and *Plodia* Gn. possess scent scales on the genitalia[1,2] and, in most species, on the forewings[1,3]. The scales are exposed only during courtship and, in *P. interpunctella* (Hubner)[4,5] atleast, removal of those on the forewing greatly reduces the probability that males will succeed in mating. Ablation has similarly been employed to demonstrate roles for scent scales of males of other lepidoptera[6-8]. With few exceptions[9-11], it has nevertheless proved difficult to extend such studies on male pheromones, largely because of an apparent lack of overt behavioural responses on the part of successfully courted females[10].

Females of *E. cautella* (Walker), however, assume an acceptance posture as a prerequisite to mating[12]. This posture often appears before the courting male has physically contacted the female[12], where it is presumably mediated by mechanical, chemical or visual stimuli acting over a short distance. We report here the experimental induction of the female's acceptance posture in the absence of males.

The study arose from a chance observation, that one of a group of calling females in a container assumed a well-developed acceptance posture when the container was moved. This suggested that relatively non-specific mechanical stimulation might be sufficient to mediate conversion of calling to the acceptance posture. Of various mechanical stimuli subsequently employed in attempts to obtain consistent and well-developed acceptance postures, an air jet proved the most successful. The jet was obtained by expelling air (flow rate 190 ml/min) from the narrow (approximately 1 mm diameter) end of a disposable pipette. The pipette was aimed from a distance (5–15 cm) at the female's head and brought towards it slowly. Females which assumed the acceptance posture did so almost always when the tip of the pipette was within 1 or 2 mm of the female's head. We therefore examined this response of females during the early scotophase ($^1/_2$–$1^1/_2$ h after dark), when sexual activity is at a peak[12,13].

Responses[14] were recorded to 2 treatments, the air jet[15] alone and the air jet which had passed over 20 sets of recently excised male wings[16]. These were offered concurrently to 2 separate groups of 10 females in 19-l containers. The antennal position and calling posture of each female were recorded immediately before and throughout the period of stimulation, and females were subsequently classified according to whether (or not) they at some time held the abdomen strongly bowed, the ovipositor exserted and the antennae fully forward. This specific calling posture, hereafter referred to as the 'fully receptive' posture, has elsewhere[13] been identified as that exhibited by early scotophase females most likely to accept courting males. Stimulation of a female was maintained until either she flew or walked away in a determined manner (the rejection response) or she assumed a well-developed acceptance posture (the acceptance response).

Results are summarized in the table. A substantial portion of those females which at some time exhibited the 'fully receptive' posture responded with the acceptance posture when stimulated mechanically by the air jet. In contrast, females which exhibited a 'less receptive' posture (i.e. did not call strongly and/or did not hold antennae fully forward) and which at no time exhibited the 'fully receptive' posture, only rarely gave an acceptance response. Similar differences were evident between the responses of 'fully receptive' and 'less receptive' females when volatiles from the male wings were incorporated with the air jet. Moreover, the proportion of 'fully receptive' females which assumed the acceptance posture was significantly greater when stimulated with the air jet containing volatiles from the male wings than when stimulated with the 'clean' air jet ($t = 3.081$ with 17 d.f., $p < 0.005$ with 1-tailed test). No such difference was evident between the responses of 'less receptive' females to the 2 treatments.

The wing scent glands would appear to have been the most likely source of the stimulatory volatiles from the male wings. Since a well-developed acceptance posture

Effect of a jet of air directed at the heads of female *Ephestia cautella* during the early scotophase

Pre-response posture:	'Fully receptive'*		'Less receptive'		
Type of response:	Rejection	Acceptance	Rejection	Acceptance	
Number of responses obtained to:					Total
Air jet only:	40	12	46	2	100
Air jet first passed over excised ♂ wings:	20	22	57	1	100

*Abdomen strongly bowed, ovipositor exserted, antennae held fully forward (see text).

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14 10 responses were recorded from each group of 10 females, every effort being directed towards stimulating individuals only once.
15 The angle at which the air jet impinged on a female's head was dictated by her position within the container, since the jet could be introduced only from above.
16 The proximal end of each male forewing had previously been sliced through to expose the bases of the scent scales normally covered by the cuticular flap.

was obtained fairly readily in response to mechanical stimulation alone, and much more readily in response to mechanical and olfactory (probably pheromonal) stimulation combined, the experiments suggest that the female's acceptance response to a live, courting male might similarly be mediated by mechanical stimulation alone, but more readily by mechanical and pheromonal stimulation combined.

In the course of attempts to refine the method of stimulation, solitary females and females in groups were both treated with the air jet. Surprisingly, no acceptance responses were recorded during 41 observations of solitary females, although 10 of these females showed the 'fully receptive' posture. During 57 observations of 'group' females, however, 15 acceptance postures were recorded, 14 of which were obtained from a total of 37 females displaying the 'fully receptive' posture. These and similar data, and detailed observations of the diel activity rhythms of solitary and 'group' females, suggested that solitary females were generally less likely than group females to assume the 'fully receptive' posture and were more likely to locomote spontaneously as well as in response to stimulation. The presence of neighbouring females evidently exerted a profound influence on the behaviour of individuals, in such a way that solitary females seemed unsuitable as candidates for the experimental treatment.

In any case, these experiments describe the basis of an assay for the identification of male sex pheromones in this and similar species. The female's response is identical with that which normally leads to mating; it is obtained fairly readily in the absence of live males by means of easily applied mechanical stimuli; it is easy to combine olfactory stimuli with the mechanical stimuli; and male wing scent scale pheromone appears to accentuate the female's response.

Isolement de deux chlamydiales (rickettsies) chez un arachnide: L'araignée *Pisaura mirabilis* Cl.
Two chlamydiales (rickettsias) in an arachnida: The spider *Pisaura mirabilis* Cl.

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Summary. 2 chlamydia-like microorganisms are associated with fatal diseases of the spider *Pisaura mirabilis* Cl. The general characteristics of these organisms are indicated and place them inside the genus *Porochlamydia* (order Chlamydiales).

Lors de l'étude de la fréquence chez l'araignée *Pisaura mirabilis* Cl. d'une maladie à *Baculovirus*[1], 2 types de particules d'allure rickettsiennes, différentes de celles notées précédemment chez cette espèce[2], ont été observées chez près de 10% des individus. Elles vont faire l'objet de cette communication.

Méthodes. Les formes infectieuses, nécessaires aux infections expérimentales, sont obtenues par broyage des cadavres d'araignées dans de l'eau distillée. Elles sont concentrées, après une clarification de 20 min à $1000 \times g$, par centrifugation du surnageant à $5000 \times g$ durant 20 min. Pour l'étude au microscope électronique, les formes infectieuses concentrées sont contrastées négativement sur grilles par du phosphotungstate de sodium neutre à 2%. L'étude histologique est réalisée sur des coupes de matériel fixé par le liquide de Helly[3], après coloration selon la technique de Mann-Dominici[3]. Pour la microscopie électronique, les coupes fines colorées par l'acétate d'uranyle et le citrate de plomb sont réalisées à partir de fragments de tissus inclus dans le mélange de Spurr[4] après double fixation par du glutaraldéryde à 3% et de l'acide osmique à 2% dans un tampon cacodylate de sodium (0,2 M, pH 7,2) additionné de saccharose (3%).

Résultats. 2 types de rickettsies (rickettsie de type I et de type II) sont reconnues au microscope électronique lors de l'examen des formes infectieuses contrastées négativement (figures 1 et 2). Celles de type I sont ovoïdes, d'aspect homogène et mesurent en moyenne 560 nm de long sur 240 nm de large (figure 1); celles de type II, de plus grande taille (en moyenne 950 nm de long sur 300 nm de large), ont un aspect hétérogène (figure 2). Les araignées, infectées expérimentalement par voie buccale avec l'une ou l'autre des rickettsies, commencent à mourir 4 semaines après l'infection. On note alors la présence de microcolonies dans les cellules de l'hèpatopancréas. En microscopie électronique, ces microcolonies apparaissent composées de divers types de particules qui traduisent l'existence d'un cycle de développement intracellulaire identique à celui des Chlamydiales[5]. Au cours de ce cycle, les formes infectieuses (corps élémentaires) se transforment en formes de multiplication (corps initiaux) dont les cellules filles évoluent vers de nouveaux corps élémentaires en fin de cycle en passant par des formes de transition (corps intermédiaires).

Les corps élémentaires (figures 1–6). Chez la rickettsie de type I, ils ont la forme d'un ovoïde aplati de 600 nm de long sur 200 nm de large et 70 nm d'épaisseur (figures 3 et 4). Chez la rickettsie de type II, de morphologie plus complexe, les sections transversales sont piriformes (figures 5 et 6); ils mesurent jusqu'à 1100 nm de long sur 250 nm de large et leur épaisseur varie de 70 nm dans la zone mince à 150 nm dans la région la plus épaisse. Ils sont dans les 2 cas limités par un ensemble pentalaminaire formé par 2 membranes unitaires accolées. Cet ensemble présente parfois un aspect perforé, identique à celui signalé chez d'autres microorganismes d'arachnides[6,7]. Le matériel nucléaire entoure le cytoplasme chez la rickettsie de type I (figures 3 et 4); il le longe en occupant la partie étroite des particules chez la rickettsie de type II (figures 5 et 6). Dans le cytoplasme, les ribosomes sont régulièrement alignés (figures 4 et 5). Une structure mem-

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