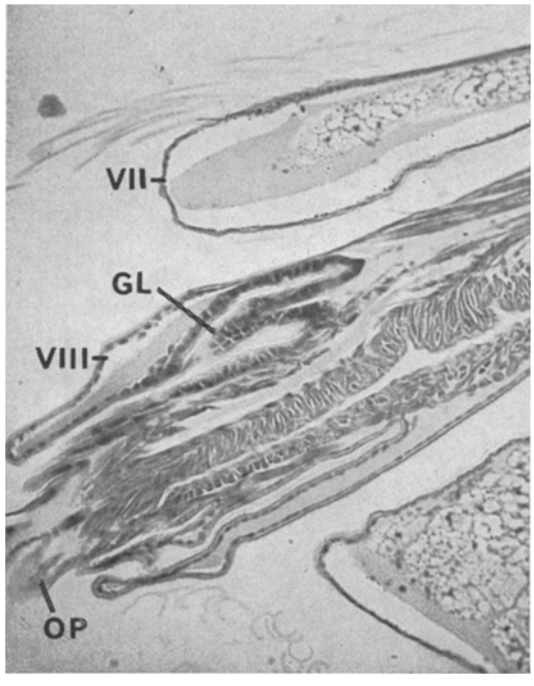
Role of Sex Pheromone, and its Insignificance for Heterosexual and Homosexual Behaviour of Larch Bud Moth[1]

The sex pheromone of the larch bud moth *Zeiraphera diniana* (Gn.) (Lepidoptera: Tortricidae, Olethreutinae) is produced by the female in a bilobate gland, invaginated dorsally of the ovipositor (Figure). The position of the gland indicates that its secretion is exposed to the air when the ovipositor is stretched out. At dawn virgin females may move along the larch twigs performing vertical vibrating movements with the tip of the abdomen and pressing it against the bark from time to time. It seems reasonable to suppose that by doing so they release the scent of the gland.

The pheromone of *Z. diniana* is a far distance attractant only, inducing males to fly towards the wind (positive anemotaxis) and thus towards the female. Near distance orientation of the males to the females, on the other hand, is strictly optical (see below). This type of behaviour may be the reason why in this species even extremely small quantities of the pheromone are still highly attractive to males in the forest[2]. However, it also makes it impossible to determine pheromone activity by a simple bioassay in the laboratory.

Concerning the chemical nature of the pheromone of *Z. diniana*, it has been shown in an earlier paper[2] that the active component of extracts of female abdominal tips has the same gas chromatographic characteristics as *trans*-11-tetradecenyl acetate (TTA) and that a wide range of concentrations of TTA (1 to 10^4 ng) are highly attractive for males of *Z. diniana*, though attractivity is lost above a certain concentration. Since traps baited with a rubber stopper containing as little as 1 ng of TTA capture even more males in the forest than traps containing 2 virgin females, and since the *cis*- isomere of TTA is a powerful antagonist of both the natural pheromone of *Z. diniana* and TTA[3], it is reasonable to suppose that the two substances are identical.



Position of pheromone gland (GL) on ovipositor (OP) of *Zeiraphera diniana* (Magn. ×100). VII, VIII indicate segment numbers.

Sexual activity of the males of *Z. diniana* is stimulated by optical signals under proper environmental conditions, i.e. decreasing light intensity at dawn, and falling temperature. These conditions have been shown generally to stimulate the activity of moths of *Z. diniana*[4]. Presence or absence of females or TTA do not influence the behaviour of the males, as demonstrated by the following experiments.

Four-day-old males were kept in groups of 20 insects in gauze cages in a room in which no females were present. The males were observed for several days. During daytime the insects were mostly sleeping, as described by MEYER[4]. Such males could not be activated by putting rubber stoppers with TTA on top of the cages. However, towards evening, when the light intensity diminished, 30–60% of the males became active and sexually excited. They started to follow each other in the characteristic manner in which they would follow a female. This behaviour was exhibited by 100% of the males if, at dawn, an upper window was opened so that a slight drought of relatively cool air was falling on the cages. Sexual excitement under these conditions may become so strong that chains of up to 7 males follow each other around the cage. Evidently the males of *Z. diniana* are not able to distinguish other males from females.

When sexual excitement is at its peak, the males try to copulate with other males and from time to time a male may succeed in catching another male with its claspers. Such homosexual copulations take place only when a chased male tries to take wing. It is then captured by the claspers of the chasing male at the very moment when it rises its wings and these, for an instant, take on the position they have in a female ready to mate[5]. This behaviour shows that, when the sexes of *Z. diniana* meet, the female has to indicate to the male that it is a female by exhibiting the specific wing position which in turn is the key factor for copulation.

Heterosexual copulation seems to depend on cooperation, be it only that the female maintains its calling position for a few moments without trying to escape. Since cooperation is lacking in homosexual copulation, the claspers of the active male cannot always catch the tip of the abdomen of the chased male but a wing or a leg only. Such homosexual copulae may last 20–40 min, whereas heterosexual copulations may last 2 and more hours. Contrary to observation on the codling moth *Laspeyresia pomonella*[6], no transfer of a spermatophore has been found during homosexual copulation of *Z. diniana*. On the other hand, homosexual copulation in the former species occurs only in the presence of the female sex pheromone, which acts as an aphrodisiac.

In *Z. diniana* neither the pheromone nor any olfactory stimulus is necessary for copulation (though males without antennae will rarely copulate with a female[7]). This has been demonstrated by treating males at dawn for 30 min

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in a box containing air saturated with formaldehyde vapour, a treatment which should completely inhibit the olfactory functioning of the antennae for at least 30 min[8]. The treated males copulated with virgin females or engaged in homosexual activity a few minutes after their release from the box.

Moreover, the sexual behaviour of groups of males under proper environmental conditions does not change in the presence of TTA or virgin females. In order to demonstrate this, 3 gauze cages with 20 males each, were put in a ventilated fume hood with dim orange light at dawn. The cages were placed side by side and separated by cardboard walls to prevent air communication. The first cage served as a control. In front of the second cage 2 rubber stoppers were placed and treated with 10^{-2} mg of TTA, whereas a cage with 5 virgin females was placed in front of the third cage. No difference in sexual behaviour could be observed in the 3 cages in two subsequent evenings. Within 1 h 4 (4) copulations occurred in the control cage, 3 (5) in the cage with TTA, and 4 (3) in the presence of females.

In a further experiment 5 virgin females were put in a cage containing 20 males. Within 1 hour 3 heterosexual and 4 homosexual copulations occurred.

The results reported in this paper show that males and females of this species will copulate independently of the presence or absence of the pheromone, if they meet each other under proper environmental conditions. Thus control of *Z. diniana* by male confusion tactics is not possible in a forest in which the population density is high enough to enable the sexes to meet accidentally, i.e. without the help of the pheromone.

Zusammenfassung. Das Geschlechtspheromon des Lärchenwicklers *Zeiraphera diniana* wird in einer Drüse des Weibchens an der Basis des Ovipositors produziert und scheint mit *trans*-11-Tetradecenylacetat identisch zu sein. Das Pheromon ist ausschliesslich Lockstoff mit Distanzwirkung und ist für die Kopulation bedeutungslos. Diese wird unter bestimmten Umweltsbedingungen durch rein optische Stimuli ausgelöst. Da die Männchen nicht streng zwischen den Geschlechtern unterscheiden können, kommen homosexuelle Kopulationen vor.

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The Diffusion Coefficient of Sodium in Barnacle Muscle Fibres

One of the unique advantages of conducting experiments with giant cells is that they can be loaded with an isotope fairly rapidly by microinjection. The technique of microinjection as adopted by me is essentially that devised by HODGKIN and KEYNES[1] for experiments on squid axons. This involves insertion of a microinjector down the center of a cannulated preparation e.g. barnacle muscle fibre, and ejection of a small volume of 'hot' solution. Some work based on the microinjection of radiosodium had been reported from this laboratory[2] but no attempt had been made to determine the diffusion coefficient of Na in the sarcoplasm. As pointed out by HODGKIN and KEYNES[1], the problem is 'one of diffusion within an infinite cylinder whose surface is insulated'. Hence the equation given by CARSLAW and JAEGER[3] can be used. The expression is:

$$\frac{y_t}{y_\infty} = 1 + \sum_{\alpha_1, \alpha_2 \ldots} e^{-\alpha^2 Dt/a^2} / J_0(\alpha) ,$$

where Yt/Y_∞ is taken as the Na efflux at time (t) relative to the maximal steady Na efflux, D the diffusion coefficient, α_1, α_2... the positive roots of the first order Bessel function $J_1(\alpha) = 0$ and a the radius of the muscle.

Twenty four experiments carried out on barnacle muscle fibres from *Balanus nubilus* or *B. aquila* were selected from a large amount of data in hand, and analyzed. These were singled out because the rate constant for ^{22}Na efflux in each instance was a constant and because the fibers were about the same in diameter (~1 mm). Computation of D from the above equation with t as 2 min gave a mean value of 2.2 ± 0.093 (S.E. of the mean) × 10^{-6} cm²/sec at 22–23° C. This is more than thrice the value reported for skinned barnacle fibers by KUSHMERICK and PODOLSKY[4] who determined the longitudinal diffusion of Na from the diffusion equation for an infinite slab. On the other hand, the value of 2.2×10^{-6} cm²/sec is half that found by BITTAR, CALDWELL and LOWE[5] in muscle fibers from the crab, *Maia squinado*. One reasonable explanation for this difference is that the T-system in barnacle fibers is more elaborate and well-developed than in *Maia* fibres (BITTAR et al.[2]; RICHARDS[6]).

As reported by BUNCH and KALLSEN[7], the diffusion coefficient of urea and glycerol in barnacle fibres is 1.87×10^{-5} cm²/sec and 1.25×10^{-5} cm/sec, respectively. This result is rather surprising not only in view of such physical factors as the T-system and SR but also the viscosity of the sarcoplasm. That retardation does take place is shown by the recent work of CAILLÉ and HINKE[8] who found the diffusion coefficients of sorbitol and Na reduced by almost 50%.

Zusammenfassung. In Muskelfasern der Entenmuschel (*Balanus nubilus* und *Balanus aquila*) beträgt der Natrium Diffusions-Koeffizient $2{,}2 \times 10^{-6}$ cm²/sec.

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