intermediate ranks of insemination order. Laidlaw and Page[4] did not observe any effect of the insemination order in their recent study and hence concluded, that there should be no sperm precedence or depletion in honeybees.

One explanation for those contradictory results from previous studies might be the uncontrolled number of sperms used for each insemination. Number of sperms were not counted or standardized prior to insemination in both studies which did use undiluted semen of drones. Therefore inter male sperm number variation may have covered the effect of extra spermathecal sperm competition. Another reason might be differences in insemination technique, which may well affect the distribution of semen in the vagina and oviducts prior to entering the spermatheca. A nonrandom distribution correlating to the packing in the syringe should be most likely. As the migration of spermatozoa into the spermatheca is a function of time[10,27], those fractions close to the spermathecal duct therefore might enter in higher numbers than semen located in the lateral oviducts. This post insemination sperm distribution may be strongly affected by the techniques used. Therefore also comparisons to natural matings should be made carefully. Up to now we do not know whether there is any sperm competition in natural matings. The present data may suggest such mechanisms but definitely more experimental data on natural matings is neccessary to rule out other hypotheses.

Nevertheless there is some evidence in the mating behavior of drones which may have evolved from 'last male advantages'. The mating sign, a part of the male endophallus, is left in the queens bursa copulatrix[34] after mating. Though at present its function during mating is unclear, it may well have evolved from a type of 'mating plug' to prevent other males from mating[29]. However in natural matings this mating plug apparently is not very efficient. The honeybee is highly polyandrous and *A. cerana* queens can mate with up to 30 males[18]. During mating drones can easily remove the mating sign of the previous one[35]. Therefore other strong selective forces at the queen or colony level are likely to counteract the desired 'last male strategy' of the drone. A selective advantage for highly polyandrous queens could be an efficient counter strategy. There are several studies and hypotheses showing that multiple mating of the queen may indeed increase individual –, colony –, and population – fitness in honeybees[36–38].

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Influence of host plant and larval diet on ovarian productivity in *Acrolepiopsis assectella* Z. (Lepidoptera: Acrolepiidae)

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Summary. Females of *Acrolepiopsis assectella*, reared on a semi-synthetic diet and laying on artificial substrates, do not respond to external stimuli by increasing ovarian production. When returned to the natural host (*Allium porrum*) for only one generation, ovarian production again rises and reaches the same level as in wild females, but its variability is strongly reduced. We conclude that selection under artificial conditions eliminates individuals which strictly depend on host plants for stimulation of larval nutrition and of reproduction.

Key words. Lepidoptera; *Acrolepiopsis assectella*; host plant; larval diet; ovarian production; stimulation; insemination.

Oogenesis in most insect species is related to environmental signals, and reserves stored during the feeding period. Stimulation of oogenesis is under the control of the brain and is mediated by the endocrine system, as has been shown for example in *Drosophila*[1]. In many phytophagous Lepidoptera, reserves are accumulated during larval life. Adults have no feeding needs;

their role is thus only to find mates and the specific host plant for reproduction. For specialized insects, substances emitted from host plants act as signals that attract adults and promote mating. These signals, and also those coming from insemination, can induce a considerable increase in oocyte number.

In *Acrolepiopsis assectella* (Lepidoptera: Acrolepiidae), which strictly depends on cultivated *Allium* plants (mainly on the leek, *A. porrum* L.), the host plant is necessary for mating to take place under natural conditions[2]. Oogenesis begins in the pupa and goes on during imaginal life. Leek given to females stimulates oogenesis in synergy with copulation[3,4] and induces egg-laying[5]. For laboratory populations, the problem of stimuli is different, breeding methods making searching for the natural host useless. Using laboratory populations bred on leek or on an artificial diet, we have studied the influence of external stimuli on ovarian productivity and its variability.

Material and methods. Three experimental populations of *A. assectella* were used. For each one, samples were taken among a great number of individuals. 1) Population W has been maintained for 10 years on fresh leek leaves, and once a year revitalized with wild insects. Laying substrates were pieces of green leek leaves. This population derives from the population W_T studied by Thibout[4]. 2) Population A was derived from W, and had been permanently selected on a semi-synthetic diet, containing leek leaf powder, for about 100 generations. Laying substrates were plastic slides coated with leek juice. The method was described by Arnault[6]. 3) Population AL was issued from larvae of population A which were fed on fresh leek from their hatching until pupation just before the experiments, in order to study the influence of food type on the productivity of these insects.

All the insects were maintained at 25°C, 14/10 h LD and 60–65% relative humidity. Two regulated chambers were used; one served only for leek-free experiments. To determine oocyte number, 24-h-old male and female pupae were weighed and the females were dissected 48 h after emergence. To compare the three populations for ovarian production, individual females (virgin or inseminated) were isolated in test tubes, in the presence or absence of a piece of leek leaf (1 × 4 cm) as laying substrate. When females were given leek, it was renewed every day. Experimental protocol was as described by Thibout[4], except that mating was always effected in the absence of leek in our case.

Ovarian production per female was calculated as the total number of eggs laid, plus the number of mature oocytes still remaining at the death of the female. This production was tested according to three factors: larval food (fresh leek or semi-synthetic diet), insemination (or absence of it), and laying substrate (fresh leek or nothing).

Preliminary results. Breeding methods eliminate all females which have not laid during four nights after mating. This has an influence on the composition of the population. On leek, all inseminated females begin to lay eggs the first night after insemination. On the plastic substrate, used for breeding of the population A, 90% of females A are able to lay eggs immediately (the night after insemination); only 25% of females W do so[7]. In population AL, fed on leek during only one generation, 95% of the females immediately start laying eggs on the artificial substrate. With no substrate, retention of eggs by the females of the three populations is almost total.

Results. 1) Pupal weight; oocyte production of young virgin females. Table 1 shows that weights of males and females are higher in the population AL than in the population A, indicating a positive effect of the fresh leek as nutrient. The mean differences (+3 mg) are identical for both sexes. Ovarian production is also higher in population AL than in population A. The degree of variation for ovarian production (CV%) is larger for A, as may result from non-optimal feeding conditions of the individuals of this population. No significant linear correlation appears between weight and occyte number for A (r = –0.15), and for AL these two parameters do not appear to be strongly correlated (r = 0.48). This confirms that, in *A. assectella,* ovarian production is not strictly related to the reserves accumulated during larval development[8].

2) Effects of host plant and of insemination on ovarian productivity. Table 2 presents mean oocyte production for four populations of *A. assectella.* Results for A and AL (boxed values) were compared by a three-way analysis of variance[9]. F parameters are given with the probability level of 0.001, and DF = (1 and 192). The effect of larval diet is highly significant (F = 118). The effect of the laying substrate is also significant (F = 37). When fresh leek is used both as food and laying substrate (population AL), it induces a significant increase in oocyte number of females, inseminated or not, compared to the population A. The interaction between food and laying substrate is significant (F = 11.6); the effect of the substrate is variable with the larval diet. Used only as laying substrate (population A), leek has no significant effect on ovarian production (Student's t-test = NS, $p < 0.05$); however, average production of inseminated females laying on leek has the greatest variability of all the samples (CV = 45%). Insemination has no significant effect on ooycte number per female, in our experimental conditions (F = NS), whatever the food and the laying substrate may be. This factor (insemination) does not interact with larval food, nor with laying substrate (F = NS).

Population W was studied only for inseminated females; W_T are results given by Thibout[4]. In population W, average numbers of oocytes are higher with leek than without laying substrate (t = 3.5), as in population AL (t = 5.8). The values for population W are statistically identical to the values for population AL in the same situations. We note a very high coefficient of variation (42%) for females W without laying substrate. These results can also be compared to those of population W_T: with leek, the mean value of ovarian production for inseminated females of population W (127.3 oocytes) is identical to the mean value for virgin females of population W_T (122.6 oocytes). Without laying substrate, we observe the same kind of similarity; 92.6 oocytes for inseminated females of W, 92.7 for virgin females of W_T. Insemination seems to be without stimulating effect on oogenesis of population W.

Discussion and conclusion. As shown by preliminary experiments on initiation of egg-laying, the population W permanently bred

Table 1. Mean weight of 24-h-old *A. assectella* pupae, ♂ and ♀. Ovarian production of the same ♀ isolated, dissected 48 h after emergence

		N	A	AL	t
Weight (mg)	♂	30	6.54 (9%)	9.47 (9%)	15.8**
	♀	30	8.24 (11.5%)	11.41 (9.5%)	12.3**
Oocytes/♀		30	78.3 (31%)	105.8 (15%)	5.2**

N = sample size; CV (%) = $\sigma/\bar{x}$; t = result of Student's t-test: ** highly significant (p = 0.001). A = population bred on semi-synthetic diet; AL = population bred on leek during one generation, just before experiment.

Table 2. Average number of oocytes produced per female of *A. assectella.* Effects of three conjugated factors: 1)larval diet: A (semi-synthetic), AL (leek for only one generation), W (leek), W_T (leek, data of Thibout[4]); 2) laying substrate: leek, or none; 3) insemination: i (inseminated), ni (non inseminated)

Laying sub-strate	Insemina-tion	Populations (larval diet) A	AL	W	W_T
Leek	i	97.0 (45%)^{ab}	144.1 (19%)^{c}	127.3 (30%)^{c}	183.7 (22%)
	ni	84.9 (29%)^{a}	136.5 (14%)^{c}	–	122.6 (21%)
None	i	82.8 (27%)^{a}	107.1 (15%)^{b}	96.2 (42%)^{ab}	114.6 (16%)
	ni	80.3 (23%)^{a}	107.5 (16%)^{b}	–	92.7 (21%)

Sample size: randomized to 25 ♀ for each situation of A and AL (boxed values); 30 ♀ for W; 60 ♀ for W_T. CV (%) = $\sigma/\bar{x}$. The means with different index letters differ significantly (Student's t-test) p = 0.001).

on leek is very heterogeneous for individual potentialities. After selection under artificial conditions (population A), almost all the females have the same behavior; they begin immediately to lay eggs on plastic with leek juice, as well as on the host plant. The situation remains the same after breeding on fresh leek during only one generation (population AL). Thus, selection seems to induce a durable homogeneity for initiation of oviposition in the laboratory populations.

We shall discuss our results on ovarian productivity, considering successively the roles of the host plant, the role of insemination, and the variability within populations.

1) Effects of the host plant. Larvae of *A. assectella* store most reserves when fed on fresh leaves of *Allium porrum*. Weight and oocyte number are higher for insects of the same strain when they are bred on leek (population AL) than on a semi-synthetic diet (population A). The population AL is able to exploit the host plant with high efficiency. Analysis of variance (table 2) shows a significant interaction: the effect of fresh leek as laying substrate depends on the previous diet. Leek stimulates oogenesis of females fed on it, whatever population they come from. Loss of stimulation of oogenesis in the population reared on semi-synthetic diet can be ascribed to a deficiency due to the absence of particular elements existing in the host plant, or to imbalances in proportions of substances. This deficiency might be linked to the larval metabolism; it might also be due to factors that affect only larval feeding behavior (physical structure and chemical composition of the diet).

The fresh host plant is necessary to *A. assectella* for high efficiency of both nutrition and reproduction. The insect-host plant specificities for these two functions do not seem to be independent. *A. assectella* is a good example of an insect which requires several stimuli for larval nutrition and for reproduction. Although successive, these stimulations are interdependent and additive.

2) Effect of insemination. In our experiments, females are always mated in the absence of the host plant. Even when bred on fresh leek, they are unable to produce additional oocytes in response to insemination. Such results differ from those reported by Thibout[4] and by Abo-Ghalia and Thibout[10] who found a stimulation of oocyte production by insemination, even in the absence of leek. We suspect that their strains have been less modified than ours by laboratory breeding. This phenomenon could be due to a lack of stimulating capacity of the males, and/or loss of inducibility on the part of the females. Stimulating effects of copulation upon oogenesis are variable among insect species[11]. We believe it is also variable among insects of the same species and depends at least partially on environmental factors.

3) Variability within populations. In wild insect populations, stimulation of ovarian production provokes a great variability in the responses of females. Because polymorphism is preserved, these insects are able to adjust to various situations, even when these differ greatly from the natural ones[12,13]. Insects of the same population do not always need the same specific stimulations. Singer[14] has described within the single species *Euphydryas editha* (Lepidoptera: Nymphalidae) several degrees in insect-plant specificity, from a generalist to a strictly specialist behavior. In *A. assectella* adapted to *A. porrum*, few females are able to lay fertile eggs in the absence of the host plant, under experimental conditions. When the situation is unusual compared to the previous breeding method, we always observe the greatest coefficients of variation. For example, females permanently bred under artificial conditions have CV = 45% with fresh leek as laying substrate; females permanently bred on leek show CV = 42% without laying substrate.

An opposite example is given by the population AL, resulting from 100 generations of artificial breeding, plus one generation on the host plant; its variability for ovarian production is the lowest of the three populations studied, whereas its average production is the highest (all the females produce more than 80 oocytes). We observed also that the heterogeneity for initiation of oviposition on artificial substrate was very low for populations A and AL, compared to population W.

We suggest alternative hypotheses: first, it can be mentioned that a constant environment may result in a lower variability[15] and may induce inbreeding and genetic drift, especially in small sized populations. But our samples of females were coming from large sized populations; in addition, the low variability for ovarian production was not observed in the population A. Thus we suppose that the lower variability is not only due to effects of inbreeding. As a second hypothesis, the results could be explained by a selective loss, during laboratory breeding, of females with low productivity, and of females that could not be brought to oviposit in the absence of the host plant. Hence we conclude that our results suggest elimination of the most strictly specialized individuals, precisely those which depend on the host plant for reproduction. Selection under artificial conditions also acts on other characters, like larval development, and induces a loss of phenotypic plasticity[6]. It appears however that a laboratory population is still able, when returned to the natural host plant, to express again most of its potentialities for nutrition and reproduction.

Variability in the physiological responses of insects to environmental factors is related to the adaptability of the individuals. Precise analysis made with isolated insects allows us to understand better the effects of controlled stimuli, in various situations. Screening the variability of insect reactions could be a useful component of studies on evolutionary interactions between insects and host plants.

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