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BEHAVIORAL ASPECTS OF MASS-REARING OF INSECTS (*)

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The present situation and objectives of mass rearing insects are reviewed under the aspect of behavior. The highest degree of "typical" behavior is required in those species that have to compete with their natural counterparts in the field. Selection pressure and conditioning during the process of mass rearing might alter the behavior of the organisms produced under artificial conditions and cause failures of field-releases. Problems that warrant more considerations are displacement failure to reach natural counterparts and the possibility of the evolution of new pest species. A sequence of actions is suggested for establishing a quality control program that elucidates the behavioral status of laboratory-reared strains.

The fact that insect behavior has been incorporated in this symposium on the implications of mass-producing insects is a symptom of the changing outlook in applied entomology in general and pest control in peculiar. It is an indication that the modern approaches to pest control — such as pest management or integrated control — have not only opened new dimensions and possibilities but have also thrown light on a few serious bottlenecks that hamper the realization of these ideas and concepts. Whereas there is general agreement that one of the fields to be investigated more intensively is ecology — especially in the area of population dynamics — the very closely related and fundamental field of behavior has hardly been touched so far. Only within the last few years have we realized that various aspects of behavior remain to be discovered that may offer excellent means for the manipulation of insect populations to man's advantage. Also we have become more aware of the fact that behavior must be considered one of the most important components in our rearing programs, one that creates major problems.

The task of covering this subject in adequate detail within the limited time available to-day is anything but easy for several reasons : First, the very core of our considerations — namely animal behavior — is a rather

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complex discipline of science and many aspects are far from being understood. Secondly, much of the information about animal behavior is available for other classes of the animal kingdom such as mammals, birds and fish. It therefore is frequently not applicable in all aspects to the problems of invertebrate behavior. Thirdly, what knowledge we do have on insect behavior has been gained from basic research on species that are not necessarily of economic importance (such as *Drosophila*). Therefore information that has a bearing on our specific problems in connection with mass-rearing and domestication are rare and have so far not been dealt with systematically.

For these reasons the present paper is far from being complete. Its main objective is to stimulate further discussion on the important aspect of insect behavior in mass-rearing which in turn might lead to improved quality control procedures.

I. Present situation

The general objective of a mass-rearing program as it can be observed in many laboratories might be summarized by the following citations from two recent handbooks :

" There is every reason to believe that it will be feasible to rear many kinds of insect parasites and predators at levels of 1000's or even 10,000's per crop acre to be released as needed for insect control... The screw-worm can be mass-produced at cost levels of about $ -.50/1000 and tropical fruit flies at less than $ -.10/1000... Regardless of the manner in which sterile insects might be employed to control or eliminate insect populations it will be necessary or highly advantageous to develop ways to rear insects in large numbers and at lowest costs possible ". (E. F. KNIPLING, 1966.)

Or : " The goal of a mass-culture program is to produce with minimal man hours and space the maximum number of fertile females in as short a time and as inexpensively as possible (" production efficiency ") " (FINNEY & FISHER, 1964).

The general emphasis as illustrated by these two representative examples is apparently put on low-cost rearing. To my knowledge there is no comprehensive paper or manual that defines and covers in detail the aspect of quality control. Test procedures for various behavioral traits have been developed in the last few years whenever problems became apparent, but most of them have either not been published or cover only limited aspects of the whole complex of behavioral and ecological problems.

In order to find out more about important behavioral aspects in a mass-rearing program let us first review the possible objectives of mass-rearing and the behavioral requirements of the insects produced.

2. Mass-rearing and behavior

Objectives of mass-cultures and importance of typical behavior.

Table 1 shows selected examples of rearing purposes and the corresponding relation to the characteristic behavior of the species.

Summarizing this table we can conclude that the importance of typical behavioral traits depends heavily on the purpose of our mass-rearing program. The highest level of " typical behavior " is required in thoses cases where the mass-reared insects have to compete with their natural counterparts in the field (intraspecific action).

Some features of mass-cultures and their impact on behavior.

Figure 1 shows a production curve that can be frequently observed when wild strains are brought to the laboratory and reared on artificial substrates (larval diet, oviposition devices, etc.). The low recovery in the first few generations or even a decline at the beginning of the rearing process followed by an increasing production after some 5-7 generations indicates that something very drastic takes place. The lack of success during the early stages of colonization suggests that the wild strain is not adapted to laboratory conditions and that intense selection occurs in the F_1 and following generations for individuals that show best results under the given circumstances. This phenomenon might be the result of a selection for individuals physiologically compa-

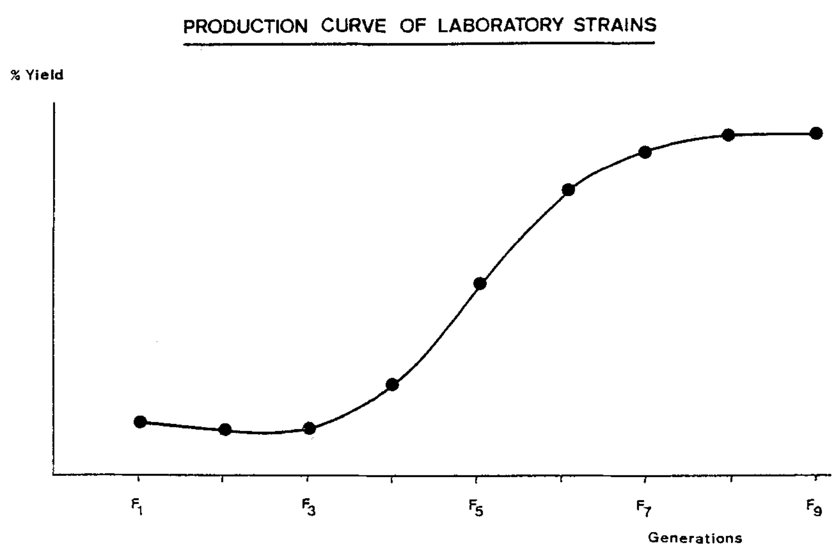

FIG. 1. Production curve of laboratory strains

TABLE 1

Objectives of mass-rearing and importance of typical behavior

Objective	Production of raw material for secondary products (virus, pheromones, etc.)	Production of hosts for entomophagous species	Displacement	Parasites and predators	Sterile-insect-technique, "Flushing" genetic suppression
Characteristics of end-product	Dead insects or living substrate	Food source Substrate	Interspecific action		Intraspecific action
Importance of high "production efficiency"	+++++	+++++	++++	++++	+++
Importance of "typical" behavior	+	+	+++	++++	+++++

tible with an artificial diet. We might also select individuals that do not show a normal oviposition behavior but accept artificial situations for oviposition and mating (such as flat membranes instead of spheres, or dropping eggs instead of pushing them into a fruit; no need for hosts for courtship and mating). Whatever the criterion might be — we force the culture genetically through a bottleneck and alter and probably reduce the level of genetic variability of the laboratory populations.

3. Some principles of animal behavior

A few words have to be said on the underlying principles of behavior which are essential for the understanding of the processes that take place during the cultivation of the insects under artificial conditions. As the science of behavior is rather complex and many aspects have to be dealt with in order to provide a sound background, I am fully aware of the risk that any simplification of this matter will cause heavy criticism from behaviorists who are experts in their field. However, as applied entomologists have to cope with the whole array of aspects of biology, we have to simplify now and then against our will and work out models that can be used in our daily work.

Figure 2 shows a simplified model of the basic components of behavior. From this model one can conclude that any change in behavior has to be analyzed as to whether it has been caused by changes in the genetical make-up of the population (e.g. by repeated selection) or by environmental influences such as conditioning or learning which result in no alteration of the genome.

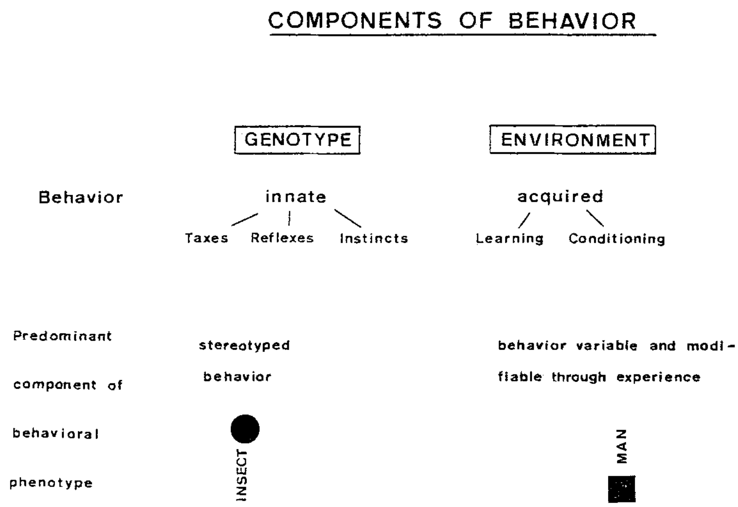

FIG. 2. Components of behavior

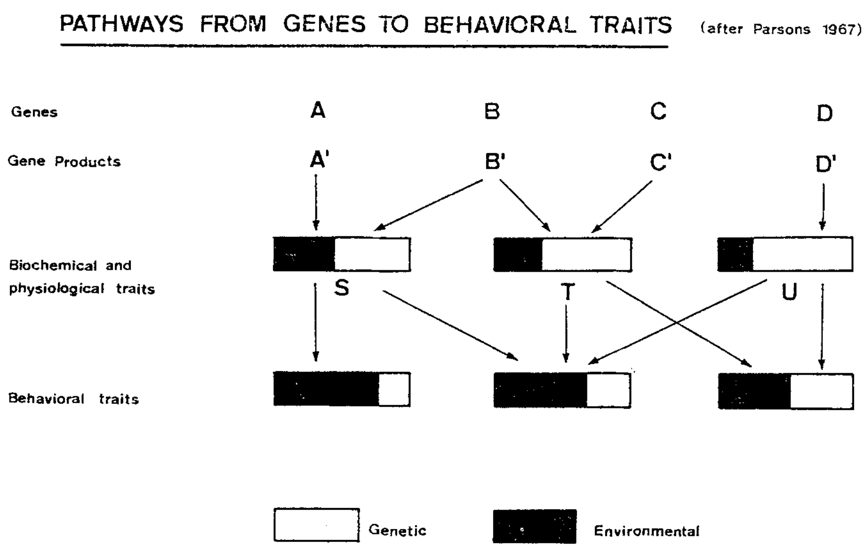

FIG. 3. Pathways from genes to behavorial traits

A model showing the pathways from genes to specific behavioral traits is given in figure 3. Single genes in most cases influence not one but several phenotypic traits in more or less obvious ways. A selection for a given behavioral trait is therefore most likely to affect other characteristics of the selected population. One example of interest is the selection for fast and slow mating speeds in *Drosophila* which will be explained further in the next section.

Summarizing this chapter on a few principles of animal behavior it is of interest that the behavior of organisms at the lower end of the phylogenetic series is predominantly governed by the genotype. Compared with higher animals, insects exhibit a much more stereotyped behavior according to the stimuli from the environment that trigger certain responses in the insect in its search for vital resources.

4. Changing behavior by selection and conditioning

Selection.

The genetic material can be manipulated by selecting individuals at either extreme of a normally distributed quantitative trait of interest in the hope of establishing specific desirable lines in subsequent generations (fig. 4a). If the trait has some genetic basis there should be a response to selection, since by selecting extreme phenotypes extreme

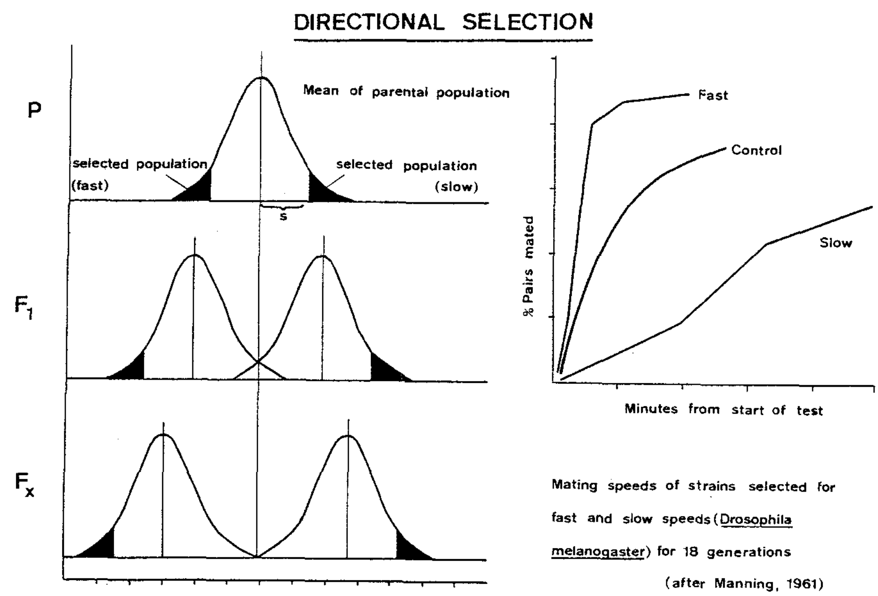

FIG. 4a. Directional selection. FIG. 4b. Selection for mating speed in *Drosophila.*

genotypes will be selected. The magnitude of the response will depend on the heritability and the selection differential S (fig. 4a).

An example is the selection for fast and slow mating speed in *Drosophila melanogaster* (MANNING, 1961). Both sexes were affected by this selection and the response to this procedure was almost immediate. After 25 generations the mean mating speed was about three minutes in the fast strains and eighty minutes in the slow lines (fig. 4b shows the situation in the 18th generation). MANNING analyzed the behavioral consequences of that selection which is of special interest in this context. Activity differences between strains were measured by admitting flies to an arena where the number of squares entered by a fly in a given time period was scored. The slow mating lines exhibited much more activity of this type, which MANNING called "general activity". Thus the fast mating lines had a high level of "sexual activity" and a low level of "general activity" with the reverse situation for the slowly mating strain. Under natural conditions these two components are presumably well balanced as over-responsiveness in either direction would be undesirable. This example might have some value for mass-rearing programs where selection for a given behavioral trait is performed sometimes unconsciously during the process of adapting insects to mass-rearing condition without considering the possible negative side effects.

Conditioning.

One of the characteristics of the feeding behavior of many phytophagous insects is the rigid specificity of diet. The origin of differences of preference has provided material for evolutionary studies and discussions for the last six decades. One mechanism which might enhance or facilitate host shifts is — besides selection as outlined above — conditioning or habituation of the preimaginal stages. Conditioning is sometimes suggested as the mechanism underlying the Hopkins Host Selection Principle, which hypothesizes that the female tends to oviposit on the plant species on which she was raised as a larva (HOPKINS, 1917).

Figure 5 shows as example the pre-imaginal conditioning of *Drosophila* for peppermint odour (THORPE, 1939; MANNING, 1967). As demonstrated in figure 2, conditioning leads to a change of behavior induced by environmental factors that does not alter the genotype of the insect as does selection. As soon as the responsible stimulus for the the conditioned trait is removed or altered in a subsequent larval generation there is also an immediate shift in the behavioral phenotype (adult) according to the new situation.

Other examples of pre-imaginal conditioning are the parasite *Nemeritis canescens* that develops normally as larva on *Ephestia* but attacks *Meliphora* after being reared on that host (THORPE & JONES, 1937) and two Lepidoptera (*Manduca sexta* and *Heliothis zea*) fed on different host plants that showed a clear preference for the plant previously eaten (JERMY *et al.*, 1968).

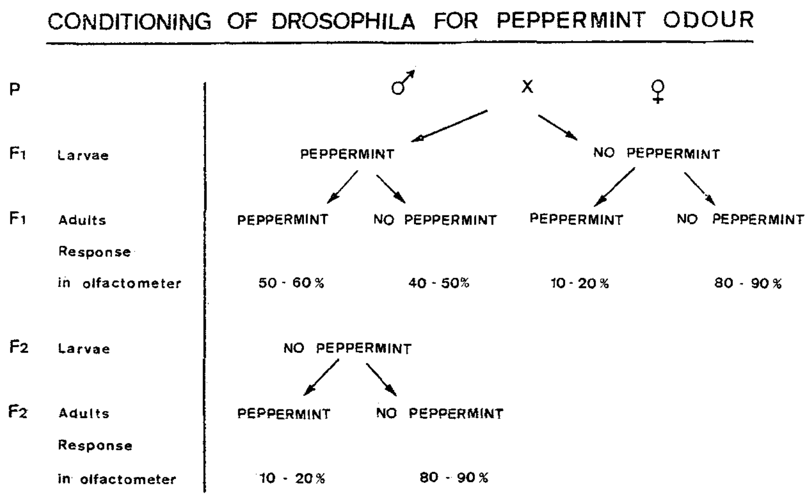

FIG. 5. Conditioning of *Drosophila* for peppermint odour.

SCHOONHOVEN (1967) showed that larvae of *Manduca sexta* grown on artificial diets are less restrictive in their plant choice than normal and will accept some usually rejected plants. This point deserves to be considered in any mass-rearing program that utilises artificial diets that do not contain host specific token stimuli. One example might be the *Rhagoletis* spp. that must find their sexual partners on their specific hosts. The absence of a token stimulus for their host might alter the orientation mechanisms of the species. This might lead to the eventual evolution of an isolating mechanism which will be further discussed in the next section.

Consequences of altered behavior in laboratory populations.

One important aspect in the mass-rearing of insects under laboratory conditions is the possibility of altered mating behavior leading to a partial sexual isolation with the wild populations. The term "isolating mechanism" was proposed by DOBZHANSKY (1937) as a name for barriers to gene exchange between sexually reproducing populations and was further defined by MAYR (1963).

Considering the various purposes for reared insects the possible isolating mechanisms can be divided into two groups. The first group includes the three important *premating* mechanisms which are of special significance for the release of sterile insects :

1. Potential mates do not meet (isolation in time and space : e.g. altered periodicities of mating activity; conditioning for other host plants with failure to find natural rendez vous sites of the sexes).

2. Potential mates meet but do not mate (ethological isolation).

3. Copulation attempted but no transfer of sperms takes place (mechanical isolation).

The second group includes *post-mating* mechanisms that can cause failure in those cases where fertile insects are to be used (e.g. increasing the native populations of parasites with released laboratory strains or use of field collected material to improve mother stocks in the laboratory) :

4. Sperms are transfered but no fertilization occurs (sperm mortality).

5. Death of zygotes occurs (hybrid inviability).

6. F_1 zygotes are viable, but partly or completely sterile (hybrid sterility).

7. F_1 hybrids are fertile, but the fitness of the F_2 or backcross hybrid is reduced (hybrid breakdown) (PARSONS, 1967).

One investigation of special interest concerning the ethological isolation which is pertinent to our discussion has been reported by EHRMAN (1964) and shall be described briefly. She studied sexual isolation between six populations of *Drosophila pseudoobscura* derived from the same initial population but maintained for several generations at different temperatures. Choice experiments showed that sexual isolation had arisen in the absence of any selection for isolation and was evidently a by-product of genetic divergence. Each laboratory population built up its own unique highly adapted gene complex with its own behavioral phenotype. Sexual isolation is a general tendency towards homogamic matings, and so may lead to a tendency of gene pools to become isolated. Within populations the term positive assortative mating is often used with a similar meaning.

The tendency of shifting towards homozygosity for various traits in laboratory populations is a major problem of inbreeding laboratory strains. Heterozygous individuals show more *behavioral homeostasis* between different environments than homozygous individuals for many fitness factors (e.g. PARSONS & KAUL, 1966, who studied the adaptation of various strains to different temperatures). Heterozygosity can be maintained e.g. by interbreeding various laboratory strains each of which shows certain advantageous behavioral traits. This is similar to the approach used in modern plant and animal breeding.

5. Selected examples of changed behavior in mass-reared insects of economic importance

Dispersal.

— *Anopheles quadrimaculatus* successfully reared in the laboratory for years by reducing the oviposition cage shows decreased flight capacity and adults failed in releasing programs (Rai, 1969).

— *Ceratitis capitata* reared in small cages shows tendency to sedentary behavior ("walking flies").

— *Carpocapsa pomonella* reared at 25° C on apple does apparently not disperse as far as natural moths at lower temperatures.

— There are indications that fruit flies (e.g. *Ceratitis capitata*) reared at optimal constant temperatures do not have the same flight capacity after they have been subjected to low temperatures (+ 6° C) for a few hours (Boller, 1971, unpublished).

Search for host and sexual partner.

— *Anthonomus grandis* males produce a less attractive sex-pheromone under crowded laboratory conditions (Gast, 1968).

— Fletcher *et al.* (1968) report altered female responsiveness of screw-worm flies (*Cochliomyia hominivorax*) to male pheromone.

— Laboratory reared mosquito males (*Anopheles quadrimaculatus*) failed to locate the females in nature (Dame *et al.*, 1964).

— There are indications that sympatric host race formation in the *Rhagoletis* group occurs within a few generations (Bush, 1969). Can the orientation mechanisms leading the insect to host tree and rendez vous site of the sexes be altered e.g. in *Rhagoletis cerasi* by artificial diets and unsuitable oviposition systems such as flat membranes and egg-dropping?

Mating behavior.

— It is suspected that the mating periods of laboratory reared codling moths (*Carpocapsa pomonella*) and of wild moths are not always well synchronized. Further studies are in progress.

— *Musca domestica* — females of three different strains mated more readily with males of their own strain (Fye *et al.*, 1966).

— Mating behavior of screw-worm flies as affected by differences in strain and size was described by Alley & Hightower (1966) and Spates & Hightower (1967). It seems that laboratory adapted male flies that fall within size-classes of screw-worm flies infrequently found in nature are unsuitable for sterile-male releases.

Oviposition.

— As to oviposition well-documented examples are rare. With various Lepidopteran species (*Carpocapsa pomonella, Clysia ambiguella* and others) it is frequently observed that wild strains brought to the laboratory show poor oviposition in the first generations.

6. Consequences of releasing insects with atypical behavior

It is a common practice that insects are mass-reared in a central rearing-plant and transported to the release sites over considerable distances. Unless the released insects match closely the behavior of the native population and are reared from strains originating from the release area, there might occur several problems which have so far not been studied systematically.

Displacement.

Monro (1966) introduced the term " flushing ", which means the overloading of the carrying capacity of the environment (food sources, etc.) with additional individuals leading to a break-down of the entire population. Crowding effects lead on the other hand to a higher dispersal rate within the populations which increased their density beyond a critical threshold. If the released insects exhibit a reduced flight capacity compared with their natural counterparts (and there is increasing evidence that this is the case in many laboratory-reared strains of fruit flies) we might speculate that a segregation of the two populations will take place at least to a certain extent. This means that the wild population might be forced to disperse over greater distances than normal and to invade other areas. The selection of a strong flying type of insect could lead to the same effect in reverse. This theoretical consideration points again to the desirable objective of rearing insects that show the *same* characteristics as wild populations with the corresponding variability (no standardized end-products).

Failure to reach natural population.

Mono- and oligophagous species might fail to find their hosts and sexual partners by altered orientation behavior due to conditioning under abnormal laboratory conditions. Incorporating the wrong or inadequate token stimuli into the larval substrate that may condition adult host selection behavior or host acceptance might have serious effects on a releasing program. Example : Where mate selection is directly tied to host selection as in the case of *Rhagoletis* spp., released flies might fail to locate wild mates.

Evolution of new pest species.

The possibility that the genetic polymorphism might be greatly altered by mixing the gene pools of two different regional strains makes it imperative that the possible effects of the hybrids produced be given careful consideration. One problem could be that the hybrids are more vigorous and adaptable than the two parent populations and might invade new ecological niches and expand drastically their distribution range. One example is the case of hybridization between *Dacus tryoni* and *Dacus neohumeralis* reported by LEWONTIN & BIRCH (1966). A second alternative is the formation of a strain that attacks new hosts eventually leading to the formation of a new pest species.

7. New objectives of mass-rearing programs

Considering all these problems centered around the behavior of the insects produced on a large scale under laboratory conditions it becomes evident that the objectives of a mass-culture as they stand to-day might no longer be valid. The highest output at lowest cost might be too expensive if the insects produced do not behave in the field in the same way as the natural populations do. Hence, we might in general agree with the statement of BECK & CHIPPENDALE (1968) who described the objective of mass-rearing plant-feeding lepidopterans as follows :

— Maintenance of an indefinitely self-perpetuating population without attenuation of either viability or reproductive capacity.

— Produce individual insects that are fully capable of competing with members of wild populations in respect to growth-rate, vigour and behavioral characteristics.

However, it is open to debate whether an indefinitely self-perpetuating laboratory population is desirable. This question has to be asked whenever we deal with uni- or oligovoltine species that normally go into diapause. When we recall fig. 1 showing a general production curve of a wild strain in various phases of "domestication", it might be worthwhile to consider a different approach. Polyvoltine species call for an instant supply of high quality insects according to the population densities of the pest in nature at a given moment. Univoltine species, however, must be overflooded with sterile insects only during a relatively short period of time each year. The question is whether it is better to expand the rearing facilities for a short period and aim at a high daily output that can probably be achieved only by a self-perpetuating non-diapausing laboratory strain that has passed through the famous genetical bottleneck and can be reared on artificial substrates. The alternative is a relatively small rearing facility that produces diapausing insects on a 52-week-basis. This latter approach might have several advantages. First, one could use to a great extent wild strains as mother-stock but is forced to develop rearing techniques that are immediately more successful than methods based on the selection of an adapted strain. Secondly, one could devote more time and attention to rearing the insects under conditions closely simulating those encountered in nature in order to maintain many important behavioral traits (e.g. host-plant token stimuli, behavior-conforming oviposition, larger cages stimulating flight activities, etc.). Thirdly, optimal temperature regimes for regulating the diapause development might allow the stockpiling of material for a considerable length of time. By doing so, it would be possible to have the necessary number of adults ready at the precise moment. In addition it would not be surprising if diapausing insects showed a behavior that comes closer to the natural one compared with insects reared continuously.

What is the production efficiency under these aspects?

Traditional concept	*Postulated concept*
" Production efficiency " : Number of fertile insects produced per monetary unit	Production efficiency : Ratio of released laboratory insects to natural insects required to reach a given objective
Criterion : Production costs	*Production costs and quality*

8. How can we tackle the problem?

The following sequence of actions is one of various possibilities :

Step 1 : *Define the problem* (behavior).

What are the essential aspects of behavior in a chronological order throughout the life-cycle of the species? (e.g. Sterile Insect Release method : Adaptation to field conditions and dispersal — finding food sources — host selection — finding rendez vous sites for the sexual partners — mating — ~~oviposition~~ — survival.

Step 2 : *Take stock.*

What is known and where do we need further information about the behavior? Make priority list according to direct impact on success. Review your quality control procedures and add new tests for essential behavioral traits.

Step 3 : Prophylatic actions when starting new colony with wild strains.

— Choose rearing methods that give relatively high yields in the F_1 generation. This avoids strong selection pressure for that small part of the population that is most adapted to artificial conditions and maintains a broad genetically determined variability.

— Do not select too early for features that lead to a standardization of your insects (" high production efficiency " on a mere cost-yield basis). This is especially important for insects that can be reared easily under the most simplified artificial conditions.

— Incorporate " luxury " stimuli in the rearing procedure that have no immediate effect on the yield but might be essential for the maintenance of certain behavioral traits (e.g. "host odour", behavioral conform rendez vous sites, etc.).

— Make the insect's life not too confortable (remote food sources to induce flight, fluctuating temperatures and humidity for maintaining a high degree of adaptability, etc.).

Step 4 : Checking " laboratory strains " (quality control).

— Perform a wide array of behavioral tests for essential traits and compare the results with the respective parameters of wild populations (not only average performance but also variability).

— Perform the quality tests not only under optimal laboratory conditions but also under the extremes similar to those occurring in the field.

Examples :

Flight: Flight capacity (flight distances, flight speed and flight pattern) with flight mills (Chambers *et al.*, 1969; Kishaba *et al.*, 1967; Boller *et al.*, in preparation).

Host finding : (especially important for oligophagous species), preference tests for various hosts.

Finding mates: Response to pheromones. Flight to artificial and natural rendez vous sites; fighting intensity between males as an indication for the proper rendez vous site.

Vigour: " General " vigour : Longevity under extreme conditions.

No stress tests (e.g. overloading of cage) because strong individuals are not necessarily exhibiting higher fitness in all aspects (Manning, 1961).

Sexual aggressiveness : SAG Test (Baumhover, 1965), copulation frequencies and time of mating, insemination rates, ratio tests (laboratory males — wild males — wild females).

Step 5 : Curative action in "abnormal strains".

General : Influence of sterilization, marking and handling procedures, etc. (HOOPER 1970).

Low flight capacity : — Increase cage size of mother stock and force insects to move actively towards vital resources (food, oviposition devices, etc.)

— Introduce wild strains or interbreed strongly flying insects from the flight mill studies (however, give careful consideration to aspects outlined in Section 7.1).

Reduced vigour :

— Make rearing conditions more natural (fluctuating temperatures and humidity, light conditions, etc.).

— Introduce wild strains to restore vigour or interbreed selected strains with desirable behavioral traits.

Step 6 : Quality control of laboratory reared strains in the field or field-cages.

Dispersal : Mark-release - recapture.

Copulation frequencies : with fertile insects in field cages with isoenzyme-method (ZOUROS & KRIMBAS, 1970) or by direct observation; with sterile insects by using markers (ratio tests) or also by direct observation.

Longevity : Mark-release - recapture.

Orientation to target (host, sexual partner, etc.) by using short range attractants (e.g. visual traps, sticky boards, sweeping. etc.)

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RÉSUMÉ

Conséquences sur le comportement de l'élevage de masse des insectes

La situation présente et les buts de l'élevage en masse des insectes sont résumés sous l'aspect du comportement. Le plus haut degré de comportement « typique » est exigé pour les espèces qui doivent exercer une action intraspécifique dans la nature.

La pression de sélection et le conditionnement pendant l'élevage en masse peuvent changer le comportement des organismes élevés dans des conditions artificielles et provoquer des échecs de lâchers en plein champ. Les problèmes qui demandent plus de considérations au sujet des lâchers sont les facultés de déplacement, l'incapacité des insectes élevés en laboratoire d'atteindre leurs partenaires naturels et la possibilité d'évolution de nouvelles espèces nuisibles. Une série d'actions est suggérée pour examiner la qualité de la production au point de vue du comportement de souches élevées en laboratoires.

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