Growth in Bupalus piniarius (Lepidoptera : Geometridae) in relation to larval population density

P. Gruys

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Dit proefschrift met stellingen van Peter Gruys, landbouwkundig ingenieur, geboren te Utrecht op 31 maart 1936, is goedgekeurd door de promotors, dr. H. Klomp, hoogleraar in de algemene dierkunde, en dr. J. de Wilde, hoogleraar in het dierkundig deel van de plantenziektenkunde.

De Rector Magnificus van de Landbouwhogeschool,
F. Hellinga

Wageningen, 29 april 1970.

Growth in Bupalus piniarius (Lepidoptera: Geometridae) in relation to larval population density

I. The influence of some abiotic factors on growth
II. The effect of larval density

Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen
op gezag van de Rector Magnificus, dr. ir. F. Hellinga,
hoogleraar in de cultuurtechniek,
te verdedigen tegen de bedenkingen van een commissie uit
de Senaat van de Landbouwhogeschool te Wageningen
op 24 juni 1970 te 16 uur

1970 *Centre for Agricultural Publishing and Documentation*

Wageningen

Aan mijn ouders

ISBN 90 220 0300 0

This thesis will also be published as Agricultural Research Reports 742, and as Verhandelingen No 1 of the Research Institute for Nature Management, Arnhem.

STELLINGEN

I

Het dichtheidseffect bij Bupalus piniarius dient te worden opgevat als een adaptatie waardoor hoge sterfte tengevolge van dichtheidsafhankelijke factoren kan worden voorkomen, en niet als een mechanisme voor intraspecifieke aantalsregulatie.

Dit proefschrift.

II

Het aantal stadia van de dennespanrups wordt op directe wijze door de daglengte beïnvloed.

Dit proefschrift.

III

Bij Lepidoptera met een variabel aantal vervellingen wordt het uiteindelijke aantal stadia vroeg in de ontwikkeling vastgelegd. Omstandigheden tijdens de embryonale ontwikkeling kunnen voor dit aantal mede bepalend zijn.

Dit proefschrift.

IV

In zijn beschouwing over de betekenis van omgevingsfactoren in de evolutie van migratie bij insekten, onderschat Southwood het belang van biotische factoren.

T. R. E. Southwood, 1962. Biol. Rev. 37:171-214.

V

Het begrip risicospreiding van Den Boer draagt niet bij tot een beter inzicht in de processen die leiden tot stabilisatie van aantallen in bevolkingen van dieren.

P. J. den Boer, 1968. Acta biotheor. 18:165-194.

VI

Het is een taak voor overheidsonderzoek om te trachten op korte termijn de vraag te beantwoorden of de gezondheid van de mens en de instandhouding van zijn omgeving meer gebaat zijn bij het gebruik van insekticiden op basis van insektehormonen en hun mimetica dan bij het gebruik van insekticiden op basis van cholinesterase remmende chemicaliën.

VII

Andere functies dan houtproduktie van bos en laanbeplantingen in Nederland zijn van zo groot belang, dat de vergelijking van het hout-aanwasverlies met de kosten van de bestrijding van plagen niet mag dienen als criterium bij beslissingen over het uitvoeren van een chemische bestrijding.

VIII

Grote specificiteit is een ongewenste eigenschap voor fungiciden.

IX

De ontwikkeling van mogelijkheden tot beperking van het aantal behandelingen met fungiciden in de appelteelt is een voorwaarde voor de praktische verwezenlijking van een beperking van het aantal bespuitingen tegen insekten en mijten.

X

Ecologisch onderzoek ten behoeve van natuurbeheer dient zich bezig te houden met de analyse van processen die zich in de natuur en in het cultuurlandschap afspelen, en met inventariserend onderzoek slechts voor zover dit ten behoeve van de analyse der processen gewenst is.

XI

De huidige omvang van de land- en tuinbouwvoorlichtingsdienst is onvoldoende om te maken dat de bestrijding van plagen op zo verantwoord mogelijke wijze kan worden uitgevoerd.

Proefschrift van P. Gruys
Wageningen, 24 juni 1970

Acknowledgments

First of all I wish to express my sincere gratitude to Prof. H. Klomp for suggesting the problem and for the stimulative discussions and criticism during the development of the work. Many thanks are also due to Prof. J. de Wilde for his close interest in the study and for many valuable suggestions. I am greatly indebted to Dr A. D. Voûte, Director of the former Institute for Biological Field Research (now amalgamated into the Research Institute for Nature Management), for the freedom he has allowed me in conducting the research as well as for many valuable discussions. I am grateful to Mr H. de Vries, who provided technical assistance throughout the investigation and whose active interest contributed to its success. Thanks are also extended to Messrs A. J. Schimmelpenninck van der Oye and M. Jacobs, who assisted in the construction of equipment. Grateful acknowledgment is made to Mrs G. Ban, Miss A. Bastiaans, Mrs J. A. Sikking, and Miss R. M. Moenen; Messrs. H. de Boer, J. Busser, E. Gruys, D. van der Ham, R. Hoefsloot, H. J. Koetsier, B. C. Kylstra, P. Leerschool, C. M. Persoons, W. A. Riemslag, C. T. van Rijswijk and B. van de Velde, without whose help it would not have been possible to perform many of the elaborate rearing experiments. I am also greatly indebted to Mrs M. H. M. Doucet, Mrs J. Overmeer, Mrs F. Joosten, and Mr A. M. Voûte, who as part of their studies conducted experiments on several aspects of the problem and kindly permitted me to use their results in this paper. I am indebted to Mrs I. Seeger for the correction of the English text; to Pudoc, in particular to Dr E. Meijer Drees, for editing this thesis and for suggesting improvements in the manuscript. I thank Miss K. Berenschot and Miss I. M. Ozinga for typing the manuscript. Finally, my deep thanks are due to my wife for her help during every stage of the work.

The present investigation was supported by grants from the National Council for Agricultural Research, TNO.

Samenvatting

In een natuurlijke populatie van de dennespanner, *Bupalus piniarius* L., constateerde Klomp (1958a, 1966) een negatieve correlatie tussen populatiedichtheid enerzijds en groei en vruchtbaarheid anderzijds.

Dit proefschrift beschrijft experimenteel onderzoek over dit verband. Het is gesplitst in twee delen. Deel I behandelt de invloed van enkele abiotische factoren op de groei van solitair gekweekte *Bupalus* larven en deel II het dichtheidseffect.

De proeven werden uitgevoerd op het voormalige Instituut voor Toegepast Biologisch Onderzoek in de Natuur (thans Rijksinstituut voor Natuurbeheer) in Arnhem, in een insektarium, het laboratorium en een kas. Tevens werden enige proeven in het veld genomen.

Deel I

Het aantal larvale stadia van *Bupalus piniarius* varieerde van vier tot acht, afhankelijk van uitwendige omstandigheden en het geslacht van de larve. Het aantal stadia nam toe bij relatief hoge temperatuur en lange dag. Wijfjes vertoonden de neiging om een maal vaker te vervellen dan mannetjes. Bij gegeven uitwendige omstandigheden was de variatie in het aantal stadia meestal niet groter dan een. In het veld maken de larven vijf of zes, en bij uitzondering vier, stadia door.

De groeisnelheid was vooral in de eerste larvale stadia positief gecorreleerd met de temperatuur, en vooral in de latere larvale stadia negatief met de daglengte. Hoge temperaturen (b.v. 25° C) en lange dag (b.v. 17 uur) hadden tijdens de latere stadia een ongunstig effect op de ontwikkeling.

Uit de resultaten kan worden afgeleid dat temperatuur en daglengte het aantal stadia rechtstreeks beïnvloeden, en niet indirect via de groeisnelheid.

De periode waarin uitwendige factoren het uiteindelijke aantal stadia kunnen beïnvloeden bleek te beginnen in het embryonale stadium en door te lopen tot omstreeks het midden van het larvale stadium.

Het endocriene mechanisme dat mogelijk het aantal stadia bepaalt wordt kort besproken.

Het gemiddelde popgewicht was positief gecorreleerd met het aantal stadia. Bij een bepaald aantal stadia, werd het popgewicht (1) groter naarmate de gemiddelde temperatuur gedurende het larvale leven hoger was, binnen de temperatuursgrenzen die een normale ontwikkeling mogelijk maken; (2) gereduceerd door kweken in het

donker. Kleine verschillen in daglengte gedurende het larvale stadium hadden geen invloed op het popgewicht.

De poppen die na een ontwikkeling met vier, vijf, zes of zeven larvale stadia werden gevormd waren morphologisch normaal. De vlinders afkomstig van larven met vier, vijf of zes stadia plantten zich normaal voort; vlinders van larven met 7 stadia werden in dit opzicht niet beproefd. De totale eiproduktie van wijfjes uit larven met zes stadia was groter dan die van wijfjes uit larven met vijf stadia. Er bestond echter geen significant verschil in het aantal gelegde eieren tussen de twee groepen, doordat de retentie van legrijpe eieren in wijfjes met zes stadia groter was dan in wijfjes uit larven met vijf stadia. In de vitaliteit van de eieren en pas uitgekomen larven van deze twee groepen werden geen significante verschillen geconstateerd.

Deel II

In laboratorium en veldproeven werd aangetoond dat de negatieve correlatie tussen larvale dichtheid en groei berust op een causaal verband. Concurrentie om voedsel speelt bij het tot stand komen ervan geen rol.

Het maximale effect van de larven op elkaar werd in de proeven reeds bij een lage dichtheid gevonden, evenals het geval is in een natuurlijke populatie.

Samen opkweken van twee of meer larven had geen invloed op de sterfte van larven en poppen, noch op de levensduur van de vlinders. In het eerste larvale stadium (L1) had het geen invloed op de groei; in alle verdere stadia behalve het laatste vertraagde en verminderde het de groei, en verminderde het de fractie van het door de darmwand opgenomen voedsel, die in lichaamssubstantie wordt omgezet. In het laatste larvale stadium was de groeisnelheid van alleen en samengekweekte larven even groot en was de verwerking van het opgenomen voedsel even doelmatig.

De gemiddelde vermindering van het popgewicht tengevolge van samen opkweken van de larven varieerde in een aantal overeenkomstige proeven van 12 – 24% bij de wijfjes en van 9 – 17% bij de mannetjes.

Bij de wijfjes werden significante individuele verschillen gevonden in het effect van samen opkweken op de groei, die waarschijnlijk het gevolg zijn van genetische verschillen in gevoeligheid voor het dichtheidseffect.

Samengekweekte larven maakten dikwijls een stadium minder door dan solitaire. Dit is waarschijnlijk een indirect effect, veroorzaakt door de vertraagde ontwikkeling van samengekweekte dieren die maakt dat zij onder een iets kortere dag opgroeien dan de solitaire.

De rupsen rustten verreweg 't grootste deel van de dag. Hun activiteit was hoofdzakelijk beperkt tot de nachtelijke periode, speciaal rond de schemering. Alleen in L1 waren de larven ook overdag actief. Er was vrijwel geen verschil in gedrag tussen alleen en samen opgekweekte larven. Alleen in L1 leken de laatste wat beweeglijker te zijn.

De chemische samenstelling van de poppen (droge stofgehalte, koolhydraat-, vet- en stikstofgehalte) verschilde niet tussen solitair en samengekweekte larven.

Het uitkomen van de vlinders werd enigszins vertraagd door samen kweken van de larven.

De vruchtbaarheid van de wijfjes werd verminderd door samen opkweken, wat te verwachten was, omdat ze positief is gecorreleerd met het popgewicht. Wijfjes uit samen opgekweekte larven legden wat kleinere eieren, produceerden meer eieren per eenheid lichaamsgewicht, en hielden minder legrijpe eieren in haar lichaam achter, dan wijfjes uit solitair opgekweekte rupsen. Maar het aantal gelegde eieren was toch duidelijk lager bij vrouwtjes uit samengekweekte larven.

Proeven over de vitaliteit van het nageslacht in afhankelijkheid van de larvale dichtheid van de ouders leverden tegenstrijdige resultaten op. Op grond van de thans beschikbare evidentie moet een eerdere conclusie (Klomp & Gruys, 1965), omtrent verminderde vitaliteit tengevolge van samen opkweken, verworpen worden.

Een analyse van gegevens van een natuurlijke populatie toonde aan dat de larvale dichtheid een veel grotere invloed op de groei heeft dan de temperatuur en de daglengte; het effect van deze laatste factoren bleek in het veld niet significant te zijn.

Uit proeven naar het mechanisme van het dichtheidseffect bleek dat de prikkel die de rupsen op elkaar uitoefenen niet bestaat uit een verontreiniging van het voedsel, en evenmin olfactorisch of visueel is. Alleen wanneer de larven direct lichamelijk contact met elkaar kunnen hebben, gedurende de nacht, blijven ze kleiner. Als ze alleen overdag samen werden gehouden verminderde de groei niet.

Vloeibare bestanddelen uit de darm, die worden uitgespuugd en kennelijk van de ene op de andere rups worden overgebracht, bleken een belangrijk bestanddeel van de prikkel die tot groeivermindering leidt. Mechanische verstoring alleen (waarop de larven overigens in gedrag op dezelfde wijze reageren als op verstoring door soortgenoten) had geen effect op de groei.

De fysiologische verwerking van de prikkel werd niet onderzocht. Wel kan worden geconcludeerd dat de prikkel een fysiologische, mogelijk endocriene, verandering moet veroorzaken die leidt tot een afname van de vreetsnelheid en de groei; rechtstreekse beinvloeding van de voedselopname, tengevolge van onderlinge verstoring, is namelijk niet de primaire oorzaak van de groeivermindering.

Zelfs wanneer het samenkweken werd beperkt tot een korte periode (b.v. een van de larvale stadia, of vijf dagen, op een totale larvale periode van 100 dagen) was een blijvende groeivermindering het gevolg, behalve wanneer het samenhouden werd beperkt tot L1.

Het aantal ontmoetingen dat nodig is voor vermindering van de groei is waarschijnlijk vrij laag, en de hoeveelheid dennetwijgen die door halfvolgroeide larven wordt doorkruist voldoende, om bij gemiddelde en hoge dichtheden in het veld een effectieve wederzijdse beinvloeding mogelijk te maken.

Het dichtheidseffect is niet volledig specifiek. Van de vier andere onderzochte soorten insectelarven uit dennebomen veroorzaakte de spanner *Ellopia prosapiaria* een groeivermindering die even groot was als die door *Bupalus* zelf teweeg wordt gebracht.

De uitdrukking van het dichtheidseffect bij *Bupalus* en het mechanisme ervan

worden vergeleken met dergelijke verschijnselen bij andere insekten, en de mogelijke functie ervan wordt besproken.

Er worden argumenten gegeven voor de veronderstelling dat hoge larvale dichtheid de neiging van de vrouwelijke vlinders om zich te verspreiden vergroot. De gewichtsvermindering ten gevolge van de hoge dichtheid zal tenminste bijdragen tot de realisering van deze neiging en zou ook een van de oorzaken van haar toename kunnen zijn. Verondersteld wordt dat de verspreiding vanuit dichtbevolkte gebieden, met een goede kans gebieden te bereiken met lage dichtheid, de kans op overleving van het nageslacht vergroot. De afname in vruchtbaarheid door de hoge dichtheid wordt beschouwd als de relatief lage prijs waarvoor een grotere winst in effectieve voortplanting wordt verkregen.

Enkele punten die betrekking hebben op deze hypothese worden kort besproken (nl. het verband tussen verspreiding van adulten en populatiedichtheid van de larven; plaatselijke verschillen in populatiedichtheid; overlevingskans in afhankelijkheid van de populatiedichtheid).

Tot slot wordt geconcludeerd dat het dichtheidseffect bij *Bupalus* niet een mechanisme is voor intraspecifieke aantalsregulatie. Het is eerder een adaptatie waardoor hoge sterfte ten gevolge van dichtheidsafhankelijke factoren wordt voorkomen, door te profiteren van dichtheidsverschillen die over uitgestrekte gebieden bestaan.

Contents

General introduction

Many external factors influence the growth and development of insects in their natural environment, and for several species population density is known to be one of these factors. When population density influences the growth of the individual, the processes involved may be competition or mutual interference (for definitions, see Klomp, 1964). The former process in particular has received ample attention in relation to the problem of population regulation. In recent years, interest in the latter process in insects as well as in other animals has grown, because it could conceivably serve as a mechanism for the regulation of population density at a low level relative to an apparent abundance of requisites. The explanation of this situation, which prevails, for instance, for the many phytophagous insects whose mean density is far below what could be expected from the food supply, is engaging the attention of several population ecologists (e.g. Andrewartha, 1959; Chitty, 1960; Iwao, 1962; Klomp, 1964; Lidicker, 1962; Uvarov, 1961; Voûte, 1957; Wynne-Edwards, 1962).

Klomp's study of a field population of the pine looper, *Bupalus piniarius* L., revealed a relationship between larval population density and growth in situations where food could not be a limiting factor. He found a negative correlation between density of the half grown larvae in the various generations studied and some parameters of larval growth (such as head width of larvae and diameter of pupae). Since pupal size is positively correlated with adult fecundity, the number of eggs produced per female decreases with increasing density of the larvae (Klomp, 1958a, 1966). An experimental analysis of the various aspects of this phenomenon seemed indispensable for an evaluation of its role in the dynamics of the population concerned. This investigation is reported in the present paper.

Part I describes growth of isolated *Bupalus* larvae, under the influence of some abiotic factors. Information about these relations is essential for the evaluation of the effect of density in the field. Furthermore, a separate treatment of this subject seemed appropriate because experimental work on it has been rather neglected in investigations on the pine looper, and because this side-line of our work provides some data of general interest with regard to growth in insects.

Part II deals with the density effect.

Part I. The influence of some abiotic factors on growth

1 Introduction

Bupalus piniarius L., whose caterpillar is know as the pine looper, is common in the Scots pine (*Pinus sylvestris*) forests in the Netherlands. It is univoltine. The pupae hibernate in the litter under the trees and the moths emerge from the end of May to the beginning of July. The females deposit their eggs on the needles in the crowns of the trees, in rows of up to 25 eggs per needle. The moths die about 14 days after emergence. About 20 days after the eggs were laid, the larvae hatch. They feed on the needles, and the full grown larvae descend to the ground to pupate in the autumn. The pupae hibernate in diapause, and resume development in spring. For more details on the life history, see Escherich (1931) and Klomp (1966).

Growth of *Bupalus*, and the effect of environmental factors upon it has received little study. It is known that the number of instars may vary and that pupal size is related to it (Bevan, Davies & Brown, 1957; Klomp, 1966). Klomp has shown that the number of instars is influenced by population density of the larvae but the impact of other environmental factors upon it is unknown. Hussey (1957), Oldiges (1959) and Schwenke (1953) have studied the influence of temperature and humidity on growth of *Bupalus*. In certain respects, it is difficult to evaluate their results: the photoperiodic conditions in their experiments are not stated, and Oldiges and Schwenke have reared the larvae in groups, the size of which is not mentioned precisely. Both photoperiod and density influence growth of *Bupalus* considerably. Hussey and Schwenke mention a variability in size of the pupae which is hard to explain from environmental conditions, and this makes it questionable whether their results would apply to our material. They do not mention variability in the number of instars.

Rather than provide an exhaustive treatment of the subject, the present study attempts to give a rough sketch of growth in *Bupalus* under the influence of abiotic factors. It is composed of the results of experiments in which larvae were reared singly under different environmental conditions. In some cases, the trends suggested by these data were further tested. The emphasis is on the pattern of growth, characterized by the number of instars, and the factors that determine it. Finally the relations found in *Bupalus* are compared with literature data on similar phenomena in other insects, and some consideration is given to the possible physiological mechanism that regulates the pattern of growth.

2 Material and methods

The experiments were conducted with offspring of pupae collected in Scots pine forests around the Research Institute for Nature Management, near Arnhem, or with the progeny of pupae obtained in previous experiments. We found no indication of a deterioration in fitness after rearing several successive generations in the laboratory.

A general account of the rearing technique is presented here; details of separate experiments will be inserted where appropriate.

Most experiments were performed in an outdoor insectary during the season in which larvae occur in the field. The insectary was placed in the garden of the Institute, where it was shaded by trees. Its roof was made of sheets of corrugated plastic painted white, and cheesecloth screens were fitted at the sides to keep out direct sunlight.

Pupae were kept in pine litter in 0.37-litre jars closed with a lid made of bronze gauze. The jars were kept in the insectary and, when emergence of moths was expected, were inspected daily. The moths were transferred to gauze cages provided with pine branches, one to four pairs per cage. Usually females were readily inseminated and deposited their eggs on the needles of the branches. The moths require neither food nor water to lay eggs satisfactorily. After the moths had died, the needles with eggs were collected and stored in Petri dishes in the insectary. They were inspected for hatching at least once a day. Larvae were reared on current-year pine shoots, in 0.37-litre jars closed with bronze gauze lids or cheesecloth. Surplus young larvae in the egg stock dishes, above the daily needs for freshly hatched larvae were killed at the end of the day. During the larval stage, food was renewed from once a week to once every other week, as conditions required. At the end of the larval period, the rearings were inspected daily for the presence of prepupae, which were then transferred to jars filled with pine litter for pupation.

In some experiments, eggs and larvae were reared under controlled conditions. Perspex hygrostats for humidity control consisted of two parts: a cylindrical rearing compartment, 12 cm in diameter and 4 cm in height, with a nylon gauze bottom and closed with a sheet of polyvinyl chloride, and a dish, which could be attached to the bottom of the rearing compartment, containing a saturated salt solution (Solomon, 1951; Zwölfer, 1932). For a rough check, humidity in the rearing compartments was estimated with cobalt thiocyanate paper and a Lovibond comparator (Solomon, 1957); the results for the different solutions agreed very well with the values in O'Brien's list (1948). Temperature was controlled by incubating the hygrostats in

thermostats or cabinets permitting a 24-hour rhythm of two constant temperatures. The insects were kept either in constant darkness or in a given 24-hour rhythm of fluorescent light and darkness, as described under particular experiments. Food was replaced once a week, and in most experiments the lower ends of the shoots were kept in small tubes filled with water.

The univoltine life-cycle was soon found to retard the progress of the work, and we therefore attempted to rear larvae throughout the year. The emergence of the moths can be advanced or retarded by a few months. Towards the end of winter, when diapause has been broken by cold, keeping the pupae at room temperature causes the adults to emerge in a few weeks (see Schoonhoven, 1962). In this way we obtained moths at the end of March. Oviposition occurred readily in a greenhouse which was heated during cold days and in which the natural daylength was supplemented with fluorescent light to provide a photoperiod of 16 to 18 hours. On the other hand, by storing pupae in a refrigerator at the end of the winter, emergence was prevented and moths were obtained in late summer, by subsequent warmth. Much longer storage of pupae at 0° to 5° C appeared to be useless, because either the moths emerged gradually during storage or the pupae died. When rearing larvae outside the period of their natural occurrence, daylength proved to be one of the main factors for normal development (Chapter 3).

Rearing of two complete successive generations in one year would be difficult, because of the slow development of the larvae and the longish cold period required to break diapause in most of the pupae (at least 10 to 15 weeks at 3° C, according to Schoonhoven, 1962). Experience showed that the cold period during the pupal stage

Table 1. Influence of temperature during the pupal stage on the moth.

Temperature (°C) in pupal stage		Percentage of		
		emerged moths	moths with defective wing expansion (in % of preceding)	pairs producing fertile eggs
1st to 8th week 9th week to emergence	0° 15°	61	6	21
1st to 8th week 9th week to emergence	0° 25°	14	100	0
1st to 20th week 21st week to emergence	0° 15°	87	11	75
1st to 20th week 21st week to emergence	0° 25°	74	86	0

About 60 individuals per series.
Identical conditions during adult stage in all series.

should be long, and that thereafter the temperature should be only moderate, to obtain a satisfactory percentage of breeding pairs and of females laying fertilized eggs (Table 1). Therefore, a separate batch was used for winter rearings, the adults of which were made to emerge in December-February after the pupae had been chilled during the summer and autumn in a refrigerator. This batch was obtained by advancing adult emergence by three months in each of two generations.

In winter, larvae were reared in a heated greenhouse with supplementary fluorescent light. They were fed with fresh shoots collected from young trees in the forest; the needles seemed to be equally suitable as food in the winter as they are in summer.

The rearing experiments were started by distributing newly hatched larvae from several moths randomly over the various series. Each series usually consisted of 40 to 60 larvae. Distribution of the jars over the available space was generally random. If efficient treatment required that jars of one series be kept together, the series were moved from place to place regularly.

Routinely, the following information was recorded: date of hatching from the egg; instar at date of food changing, as ascertained from the shed head capsules; date of the prepupal stage (the precise date of pupation was not ascertained to avoid disturbance of pupating larvae). In most experiments, had capsules were collected for measurement of their largest width with the eyepiece micrometer of a binocular microscope. For the pupae, sex, weight, and greatest width (measured with an eyepiece micrometer) were determined. Special observations and measurements will be described in particular experiments.

Unless otherwise stated, two-tailed tests were applied at a level of significance of $\alpha = 0.05$. Since most of the experiments were designed to assess particular effects, relevant differences between pairs of treatments were tested separately in experiments consisting of more than two treatments instead of applying range tests (Pearce, 1965).

3 Results

3.1 Number of larval instars in relation to environmental factors

The number of larval instars varied from five to six in rearings in the insectary during the summer. Under various laboratory conditions, pupation occurred at the end of either L4, L5, L6, or L7. In a few cases, the larvae even entered an eighth stage, but death intervened before their pupation. Under any conditions, the variation in the number of moults was generally not more than one.

Sex The number of larval instars was related to sex. If conditions permitted variation in the number of instars, a significantly greater percentage of females than of males showed the higher number (tables 3, 5, and 6; figs 1 and 2).

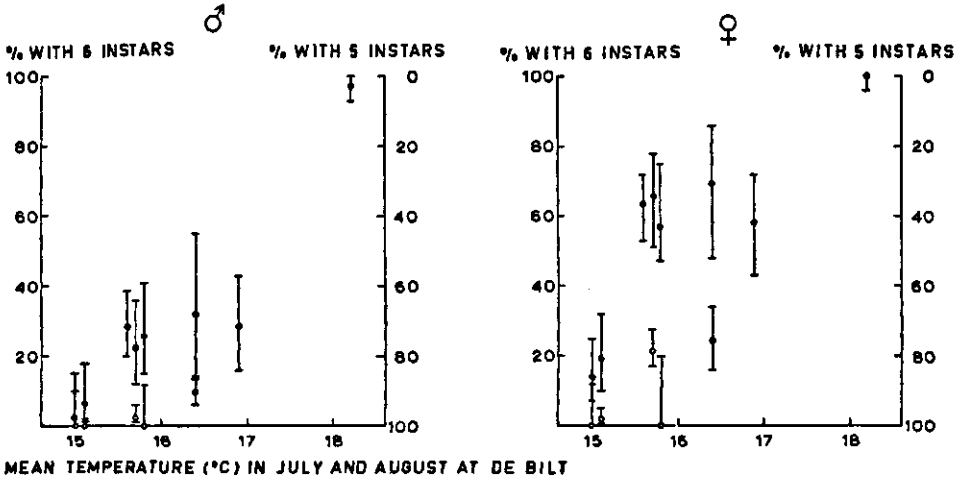
Temperature During larval development, temperature has a marked influence on number of instars. Evidence for a positive correlation between temperature and number of moults is provided by Fig. 1. Because the temperature in the insectary was not recorded in all years, the mean temperatures used for the graph were obtained from the Royal Netherlands Meteorological Institute in De Bilt, 60 km from the laboratory. They provide a satisfactory basis for ranking the different years in order of temperature (Table 2). July and August were taken because the fourth instar is generally completed by the end of August and the ultimate number of instars may be expected to have been induced by then, as will be shown in Section 3.3. Data for larvae hatched early and late are shown separately, because daylength and therefore date of hatching, also influenced the number of instars.

Table 2. Monthly averages of daily temperatures (in °C) in the insectary and in De Bilt in the summer of 1961.

	Insectary			De Bilt		
	mean	maxima	minima	mean	maxima	minima
June	15.6	22.7	10.4	15.4	20.3	9.9
July	15.5	21.0	11.5	15.6	19.7	11.3
August	15.7	20.4	11.8	16.0	20.1	11.6
September	16.8	20.8	13.1	16.6	21.3	12.6

Fig. 1. Relation between mean temperature during July and August, in eight years, and number of instars of larvae reared singly in an outdoor insectary.

Dots: larvae hatched from 19 June to 6 July.
Circles: larvae hatched from 6 July to 29 July.
95% confidence intervals are given.
Correlations for early hatchings are significant (Kendall's test).



The results of the experiments under controlled conditions corroborate the effect of temperature. Table 3 shows the marked difference in development caused by temperature regime, in larvae reared in constant darkness (except for the changing of food, when the larvae were taken into the light). At moderate temperature, pupation occurred at the end of L4, and in warmth at the end of L5. Similar results were obtained in two other experiments (Table 5, Series 1 *vs.* Series 5; Table 26).

Humidity The influence of humidity on the number of moults, if any, is contradictory (Table 3). It was not tested over a greater range.

Daylenght The prevalence of larvae with four instars at about 16° C in constant darkness, as compared to the absence of such larvae at about the same temperature under natural summer daylength (Fig. 1), points to an influence of daylength on the number of moults. Such an effect is also strongly suggested by the data in Fig. 2, which shows the development of four batches of larvae hatched at different dates in the summer of one year and reared in the insectary. Early and late hatching was achieved by shifting the period of emergence of the moths (Chapter 2). In the early rearings, six instars prevailed, the picture changing gradually until the last group to hatch, in which larvae with four instars were in the majority and those with six were absent (see table on right of Fig. 2). Pupation occurred during about the same period for all groups, which means that the larval stage was extremely prolonged in

Table 3. Influence of temperature and humidity on the number of larval instars in singly reared larvae, in constant darkness.

Series	Temperature (°C)		Relative humidity %	Percentage animals with			
	08.00 hr to 20.00 hr	20.00 hr to 08.00 hr		4 instars		5 instars	
				♂	♀	♂	♀
1	18	10	77	89	76	11	24
2	18	10	94	94	89	6	11
3	24	20	77	39	19	61[1]	81
4	24	20	94	19	5	81	95

1. Including one larva with six instars.
Gradual change between day and night temperatures.
Effects of sex and temperature are significant; effect of relative humidity at 18–10° C: P = 0.17; at 24°–20° C: P = 0.02.
About 70 larvae per series.

the early group and extremely shortened in the late group, as compared to the normal development of the batches that hatched in between. The position of the middle of L3 on the time axis indicates that it was the duration of the second part of larval life that underwent the most change.

Temperature could hardly be the cause of the difference between the four groups in the number of instars. The larvae in the early series were exposed to a lower average temperature during their first three stages than those in the groups that hatched afterwards, but passed through the highest number of instars (Section 3.3: the first three or four stages are critical for the induction of the ultimate number of moults). A similar line of reasoning applies to the late group. Therefore, daylength rather than temperature was the most plausible cause of the observed differences, long days causing the larvae to pass through more instars and to prolong the later stages, and short days resulting in the opposite.[1] The same relation between number of instars and date of hatching is found in Fig. 1 (circles against dots). Further remarks on rate of development in relation to daylength and temperature will be given in Section 3.5.

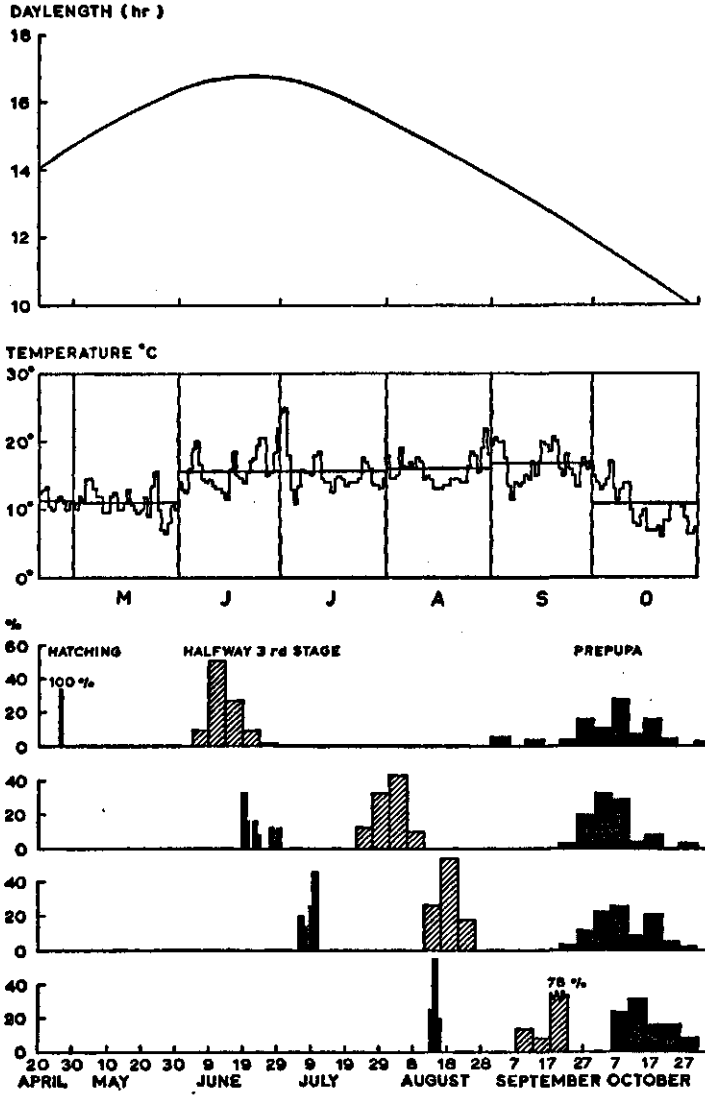
Further evidence for the influence of daylength on the pattern of growth was found in an experiment in which the larvae were divided into two groups immediately after hatching, one group being reared under long-day conditions (17 hours of fluorescent light) and the other in constant fluorescent light. This experiment was

1. Long photoperiod is not necessarily the only or main cause of the long duration of the second part of the larval stage in the first Series of Fig. 2. During L1, L2, and part of L3, these larvae have been exposed to rather cool conditions which may, as Hussey (1957) has found, prolong the later stages.

carried out in an unheated greenhouse; temperature was not controlled but was roughly the same for both series, as judged from thermograph recordings. Because a long day (and constant light) seemed to impede development in L4, the photoperiod in the long-day series was gradually shortened in L5 to 12 hours. In the con-

Fig. 2. Relation between date of hatching of the larvae and the number of larval instars, and rate of development.

About 50 singly reared larvae per group. Temperature (recorded in insectary): daily and monthly averages. Daylength: from sunrise to sunset. All differences between successive groups in the table are significant.



NUMBER OF INSTARS	♂			♀		
	4	5	6	4	5	6
	(%)	(%)	(%)	(%)	(%)	(%)
	0	52	48	0	23	77
	0	74	26	0	43	57
	0	100	0	0	100	0
	92	8	0	52	48	0

stant-light series no change was made, so that development was greatly protracted and less than 10% of the larvae pupated. Table 4 shows the results, starting with the number of larvae in L4. Because most of the larvae in constant light died before pupation, the number of larvae moulting to the next instar is presented together with the number of dead or pupating larvae in each instar. Larvae moulting to L7 occurred in both series; under normal daylength, however, they were absent (cf. Fig. 1; in the present experiment, the average temperature in the period from hatching to the end of L4, which corresponds to July and August in Fig. 1, was 17.9° C). The number of moults was significantly higher in constant light than in the long-day treatment shown in Table 4.

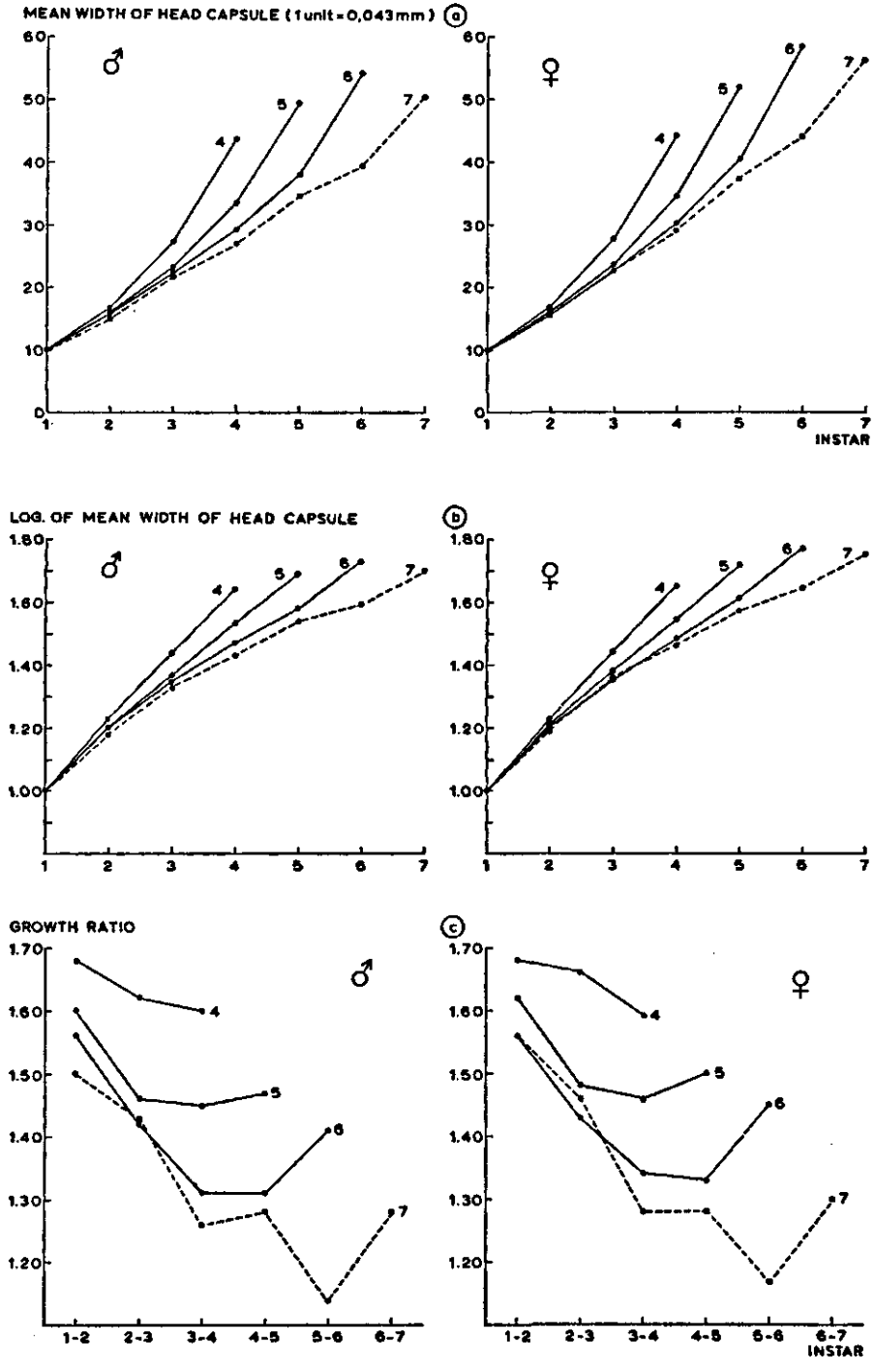
Since four-instar development occurs in insectary rearings of larvae hatched late in the season (Fig. 2, Series 4), and because such late hatching may well take place in the field in some years, a four-instar development in a certain proportion of the larvae in the field seemed probable. This was investigated in 1962, after a late and cool summer, among some hundred pine loopers collected from a nearby forest on 20 September and reared singly in the insectary. The head widths were measured at collection and during any subsequent stage(s). From these data, the number of instars could be inferred, with certainty for most individuals, by reference to the

Table 4. Influence of daylength on the number of larval instars in singly reared larvae.

	Long day			Constant light		
	number of larvae	% of number in L4	number of dead larvae (d) and of prepupae (pp) in indicated instar	number of larvae	% of number in L4	number of dead larvae (d) and of prepupae (pp) in indicated instar
Larvae in L4	122	100	L4: 10 d, 2 pp	67	100	L4: 5 d
Larvae moulting to L5	110	90	L5: 6 d, 29 pp	62	93	L5: 7 d
Larvae moulting to L6	75	61	L6: 1 d, 65 pp	55	82	L6: 23 d, 3 pp
Larvae moulting to L7	9	7	L7: 9 pp	29	43	L7: 24 d, 2 pp
Larvae moulting to L8	0	0		3	4	L8: 3 d

For statistical evaluation, dead larvae have been taken as having pupated at the end of the stage in which they died. The difference between the two treatments is significant (χ^2-test).

Fig. 3. Growth in head width of singly reared larvae with four, five, six, and seven instars.
Males with four to six instars: points represent the means of 123–786 head widths.
Females with four to six instars: points represent the means of 81–735 head widths.
Larvae with seven instars: points represent the means of 5–10 head widths, dashed line indicates low number of measurements.



frequency distributions of head width in the different growth types. Of the females, at least 20% (and at most 24%) and of the males, at least 33% (and at most 46%) developed through only four instars; the rest passed through five. Because nearly all the larvae were in their penultimate instar at the date of collection, it can safely be assumed that the final number of instars had already been fixed in the field and that the four-instar development was not induced in the insectary after collection; as will be shown in Section 3.3, the number of instars is induced in an early stage. Nine of the collected larvae failed to pupate before winter and slowly died. All of them were in their fifth instar. This suggests that the ability to develop through four instars has survival value when hatching has been relatively late. In this connection it is of interest to notice that in England, where four-instar development occurs in the field (Crooke, 1956), the phenology of the pine looper is retarded as compared with that in the Netherlands; contrast Bevan *et al.* (1957), Crooke (1956) and Davies (1962) with Klomp (1958b, 1966).

Larval density The influence of larval density on the number of moults is discussed in Part II.

3.2 Larval growth

Larval growth was analysed on the basis of changes in the width of the head. For this purpose all head widths of singly reared larvae determined in the course of the investigation were pooled according to instar. The means of each instar are shown separately in Fig. 3a for eight categories of larvae, namely males and females with four, five, six, and seven instars. The increase of head width showed distinct patterns associated with the ultimate number of instars. Larvae with a lower number of instars grew faster than those with higher numbers, the difference becoming greater at each moult; but the final head width was greater, the higher the number of instars.

In the statistical evaluation of the original data, the seven-instar type was ignored because of the small number of observations. When the other growth types were contrasted, the differences in mean head widths of a particular instar proved to be significant in L2 to L5. In L1, they were not significant.

In many insects, growth follows a geometrical progression ('Dyar's law'; see Wigglesworth, 1965). Figures 3b and 3c, in which the logarithm of the mean head width and the growth ratio (i.e., the ratio of the means for two successive instars) are plotted against the number of the instar, show to what extent Dyar's law applies to the different growth patterns of pine loopers. If head width followed a geometrical progression, the points in Figure 3b would lie on a straight line, and those of Figure 3c on a horizontal line. Only for larvae with four instars does this hold fairly well; those with five instars, however, show a relatively high growth rate from the first to the second instar and a clearly lower one after that. Larvae with six and those with seven instars deviate still further from a geometrical progression. Similar deviations from a constant growth ratio in insects with a variable number of instars have been

described by several authors (e.g. Eidmann, 1962; Harries & Henderson, 1938; Peterson & Haeussler, 1928).

It may be asked whether growth of larvae with the lower number of instars is still the most rapid when the duration of the various stages is taken into account. This question is answered by Figure 4, in which the logarithm of the mean head width of female larvae reared under three different sets of conditions is plotted against the mean age of the larvae at the beginning of each stage. The slope of the curves provides a measure for the rate of development. Again, it appears that within each set of conditions the higher number of moults was associated with the slower growth.

Some other information can be gathered from this graph. Firstly, growth was

Fig. 4. Relation between number of instars and rate of development in female larvae reared singly under three different sets of conditions.

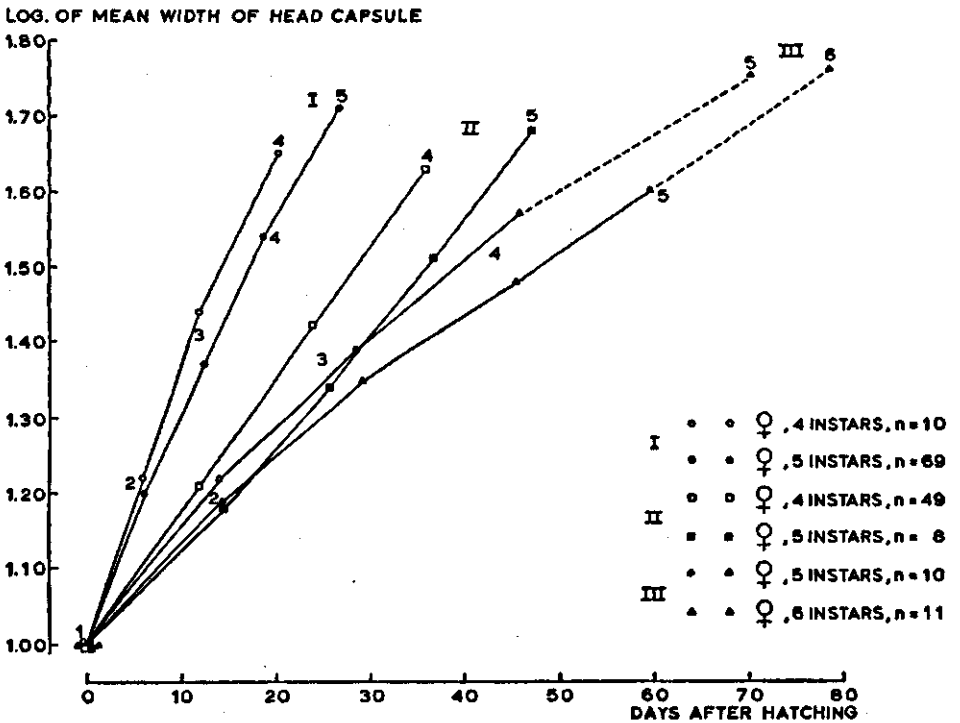
Arabic numbers indicate instars, Roman numbers conditions, as follows:

I Series 3 and 4, Table 3: constant darkness, 24° from 08.00 hr to 20.00 hr and 20° from 20.00 hr to 0.800 hr.

II Series 1 and 2, Table 3: constant darkness, 18° from 08.00 hr to 20.00 hr and 10° from 20.00 hr to 08.00 hr.

III Insectary, June-October; photoperiod 17-11 hours; average temperature 15.6°–11.0° C. Last instar head width estimated from head width of penultimate instar and appropriate growth rate, taken from Figure 3.

n = number of measurements.



slower at the moderate temperature than under warm conditions (contrast I with II), and it was faster in constant darkness than at the natural photoperiod during summer (contrast II with III; for further comments see Section 3.5).

Secondly, speed of growth evidently does not determine the number of instars, since larvae with five instars, for example, occurred in each of the three series in Figure 4, in spite of considerable differences in the rate of growth.

Thirdly, the larvae reached a larger size at the higher temperature: when comparing larvae with the same number of instars, those of series I had a significantly larger head width in their final instar than those of series II (Yates' test).

3.3 Developmental stage at which the number of instars is fixed

A comparison of the trends of the curves of the head width in Figure 3 shows that the ultimate number of instars is probably fixed in a relatively early stage, the earlier, the lower the final number of moults. All curves start at the same point (i.e. about 10 units) in L1. The curve of larvae with four instars begins to diverge immediately, the difference in head width between this category and the others being clearly marked in L2 and becoming progressively greater in L3 and L4. Consequently, whether the ultimate number of instars will be four or more seems to be determined during L1 and L2, at least for the great majority of larvae; some, however, might still switch in L3 from a development through four instars to one through five, as was suggested by a slight amount of overlap of the frequency distributions (not shown in this paper) of the fourth instar head width. The same kind of reasoning, applied to larvae with five as contrasted to those with more instars, suggests that in that case the number of moults may already be determined in L1 in some larvae (the small difference in head width of L2 between larvae with five and those with six instars was significant) and in L2–L4 in most of them.

This conclusion was corroborated experimentally for conditions permitting the larvae to develop through either four or five instars. The experiment was based on the finding that rearing in constant darkness causes nearly all larvae to develop through four instars at 16° C and through five at 24° C. To see during which stage a temperature of 24° C induces the extra instar, three batches of larvae were reared at a basic temperature of 16° C but kept at 24° C in L1 (Table 5, Series 2) or in L2 (Series 3) or in L3 (Series 4). As controls, one batch of larvae was kept constantly at 16° C (Series 1) and another at 24° C (Series 5). The results show that the extra instar was most readily induced during L2, and significantly less so during L1 and L3. This result cannot be caused by differences in the length of the larvae's stay at 24° C, since the durations of L1, L2, and L3 were about equal, namely 6.3, 6.0, and 7.0 days, respectively. In females, the second stage alone was long enough for the induction of an extra instar, but males, which are less apt to have an extra moult under any condition, required a longer period (contrast Series 3 with Series 5). In L3 (Series 4), an extra moult could still be induced in a considerable proportion of the female larvae, but in the males, the proportion of larvae with five instars was only slightly

Fig. 5. Histograms of head widths (one unit = 0.045 mm) in L1 – L4 for larvae with 4 instars and with 5 instars (shaded) reared at 16° C or at 24° C.
Histograms of head widths laid down at 24° C are enclosed in heavy lines.
1 and 5: controls. Compare Table 5.

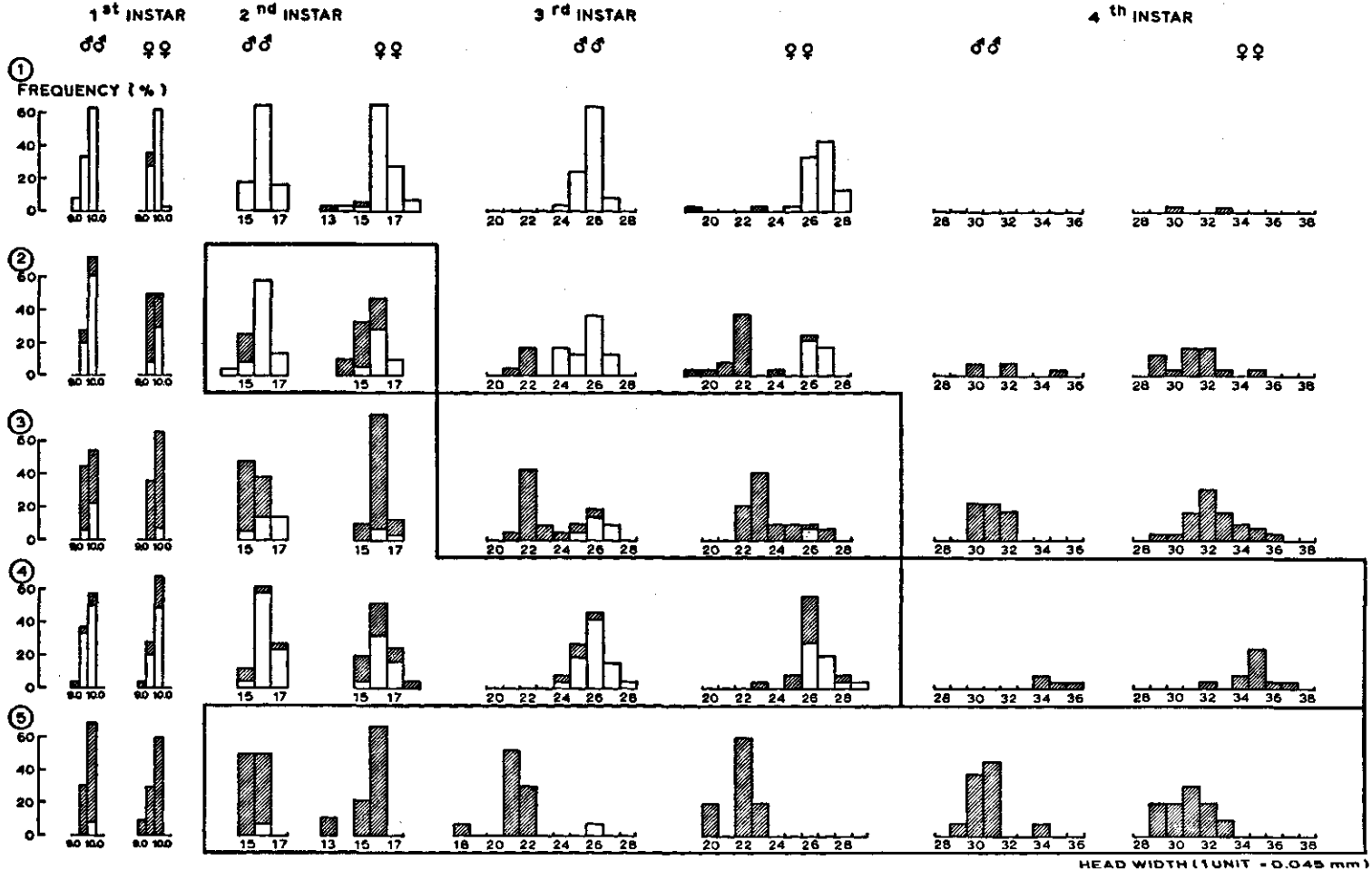


Table 5. Larval stage in which the determination of the number of instars is induced by temperature.

Series	Temperature combinations (°C)					% males with		% females with	
	L1	L2	L3	L4	L5	4 instars	5 instars	4 instars	5 instars
1 control	16	16	16	16	16	100	0	94	6
2	24	16	16	16	16	74	26	39	61
3	16	24	16	16	16	33	67	9	91
4	16	16	24	16	16	85	15	56	44
5 control	24	24	24	24	24	6	94	0	100

About 50 singly reared larvae per series.
Constant darkness; relative humidity 77%.
Differences between series are significant (χ^2-test), except between series 1 and 4, males, and series 2 and 4, males and females.

and insignificantly higher than in Series 1. As to the first larval stage (Series 2), it may be concluded that either the induction of a fifth instar occurred less readily in this stage than it did in L2 or, if the induction of an extra instar had occurred in a high proportion of the larvae, the situation was disturbed by the larvae's return to the moderate temperature in the – rather critical – second stage.

A further study of the head widths (Fig. 5) shows that the direct influence of temperature is negligible as compared to the effect of growth pattern on head size. The frequency distributions for L3 in the various series illustrate this point. Larvae on their way to pass through four instars (the unshaded columns) had about the same head size in all series, regardless of whether the third instar head capsule is laid down at 16° or at 24° C. The same applies to larvae engaged in a five-instar developmental pattern (Series 2 as compared to Series 5, shaded columns)[1].

The moment at which the ultimate number of instars is fixed affects the head width in the later instars. When an extra instar is induced early in the larval stage, the head width of the following instar is smaller than when the moment of its induction is late. This is demonstrated in the head width of L3 (Fig. 5; five-instar larvae of Series 3 are significantly larger than those of series 2 and 5) and even more clearly in the frequency distributions of L4, the means of which show a significant downward trend from Series 4 (late induction, in larvae with large third instar head capsules normal for a development through four instars), over Series 3, to series 2 and 5 (early induction).

1. The differences between series 1 to 5 with respect to the third instar head capsule of larvae developing through four instars are not significant; this also holds for the difference between series 2 and 5 in third instar head width of larvae with five instars (χ^2-test on means, and Yates' test, respectively). The differences in third instar head width between the two growth types are too pronounced to require testing.

Mortality, which did not differ between the various series in the early larval stages, was exceptionally high in the last stage of Series 5, amounting to 50% in this series as compared to 3% in series 1 to 4. Since Series 5 was kept at 24° and the others at 16° C during the last larval stage, this finding points to a downward trend of the optimal temperature for development towards the end of larval life (see Schwenke, 1953).

Accidentally, I obtained evidence that the embryonic stage is already susceptible to the determination of the number of instars by external factors: part of a batch of larvae obtained from eggs kept at a low temperature to delay development passed through one less instar than the number expected from the rearing conditions during the larval stage.

This indication was corroborated by keeping batches of eggs at temperatures of 25° or 15° C, and rearing the larvae at 25° C in constant darkness, which normally causes a development through five larval instars. The moderate temperature during the egg stage brings about a reduction in the number of instars, especially in the males; in the females, slight differences in the same direction occur, but they do not reach the level of significance (Table 6, series a1 and a3; series b1 and b4).

To obtain an impression of the susceptibility of various periods of the embryonic stage with regard to factors inducing the determination of the number of instars, a division of the egg stage in two, rather arbitrarily chosen, periods was made. The first period ended, and the second one began, on the day the larval structures became visible under a binocular microscope, the appearance of the stemmata being taken as the main criterion. At a constant temperature (of either 25° or 15° C) the duration of the first and the second periods showed a ratio of about 3 : 2. Comparison of series b1 and b2, and of b1 and b3, in Table 6 shows that during each of the two periods the embryo was susceptible (the difference between series a1 and a2, Table 6, is in the same direction, although it is not significant). A moderate temperature throughout the egg stage was possibly more effective in producing fewer instars than a moderate temperature during either of the two parts of it (series b2 *vs.* b4 and series b3 *vs.* b4), but these differences were not significant.

A temperature of 10° C, which is close to the lower threshold for embryonic development, gives no greater effect than 15° C (Table 6; series a3 *vs.* a4).

Since conditions during the early larval stages have a pronounced influence on the number of instars, it is to be expected that an induction of four larval instars during the egg stage could be affected by conditions conducive to five instars during the larval stage. The results of Series b5 (Table 6), in which not only the egg stage but also L2 was spent at 15° C, might be interpreted as evidence for this consecutive induction: as compared to Series b4 (15° C during the egg stage only), a significantly higher percentage of the larvae went through four instars.

Further evidence for a change in the growth program during the larval stage can be obtained from an analysis of the head widths of these experiments. A shift from a four-instar to a five-instar program will be revealed by the head widths of larvae that eventually pass through five instars. The head widths of L2 seem particularly

Table 6. Influence of temperature during the embryonic stage on the number of larval instars.

Series	Temperature (°C) in			% males with			% females with		
	egg stage		larval stage						
	1st part	2nd part		4 instars	5 instars	6 instars	4 instars	5 instars	6 instars
a 1	25	25	25	0	100	0	0	92	8
a 2	25	10	25	18	73	9	10	80	10
a 3	15	15	25	41	59	0	10	90	0
a 4	15	10	25	47	53	0	18	82	0

Series	Temperature (°C) in					% males with		% females with	
	egg stage		larval stages						
	1st part	2nd part	L1	L2	L3 to pupa	4 instars	5 instars	4 instars	5 instars
b 1	25	25	25	25	25	8	92	11	89
b 2	25	15	25	25	25	37	63	15	85
b 3	15	25	25	25	25	32	68	0	100
b 4	15	15	25	25	25	55	45	26	74
b 5	15	15	25	15	25	93	7	57	43

About 30 (a) and 55 (b) larvae per series. Constant darkness; relative humidity 77%.
Significance of differences (χ^2-test or Fisher's test):
a: 1 *vs.* 3 ♂♂ P = 0.01
1 *vs.* 2 ♂♂ and ♀♀, 1 *vs.* 3 ♀♀, 1 *vs.* 2 + 3 + 4 ♀♀, 3 *vs.* 4 ♂♂ and ♀♀ *not significant.*
b: 1 *vs.* 2 ♂♂ P = 0.04
1 *vs.* 3 ♂♂ P = 0.08
1 *vs.* 4 ♂♂ P = 0.001
3 *vs.* 4 ♂♂ P = 0.15, ♀♀ P = 0.004
4 *vs.* 5 ♂♂ P = 0.003, ♀♀ P = 0.04
1 *vs.* 2 ♀♀, 1 *vs.* 3 ♀♀, 1 *vs.* 4 ♀♀, 2 *vs.* 4 ♂♂ and ♀♀, 1 *vs.* 2 + 3 + 4 ♀♀ not significant.

suitable for this analysis, because this is the youngest instar showing clear differences between the mean head widths of four and five-instar larvae (see Fig. 3a). If the larval stage is started with a four-instar program and a shift to five instars is induced in L2 or later, the head width of L2 should be representative of the four-instar growth type and hence be relatively large. Such development occurring in a fair proportion of the five-instar larvae of a series, would result in a relatively high mean head width of L2.

In fact, the mean head widths showed this trend: those of series 2, 3, and 4 lied between the means of Series 1 (in which no shift from a four to a five-instar type of development is to be expected) and the mean of four-instar larvae (Table 7).

Table 7. Mean head width of L2 in the experiments shown in Table 6, and an estimate of the percentage of larvae with a four-instar growth program at the end of L1.

	Mean head width of 2nd instar (1 unit = 0.043 mm)		% individuals with a 4-instar program at the end of L1.	
	♂♂	♀♀	♂♂	♀♀
5-instar larvae, Series a 1	14.8	15.2	0	0
5-instar larvae, Series a 2	16.5	16.3	70	50
5-instar larvae, Series a 3	16.3	16.7	78	71
5-instar larvae, Series a 4	16.4	16.9	81	81
4-instar larvae, all series	17.2	17.4		
5-instar larvae, Series b 1	15.5	15.7	8	11
5-instar larvae, Series b 2	15.4	16.0	37	41
5-instar larvae, Series b 3	15.7	16.0	46	30
5-instar larvae, Series b 4	15.9	16.3	73	70
4-instar larvae, all series	16.5	16.7		

From the means a rough estimate of the proportion of larvae with a four-instar program at the end of L1 has been calculated. Example-calculation for males in Series a2 of Table 6:

Mean head width of L2, ♂♂ with 5-instar growth program from beginning of larval stage (5-instar ♂♂ of Series a1) 14.8 (1)

mean head width of ♂♂ with 4-instar growth type 17.2 (2)

(2) - (1) 2.4

mean head width of 5-instar ♂♂ in Series a2 16.5 (3)

(3) - (1) 1.7

percentage ♂♂ with 4-instar program at end of L1 (see percentages in Table 6, a):

$18 + (1.7 : 2.4) \times 73 = 70$

It should be mentioned that this line of reasoning is only valid if temperature during the egg stage influences head width of L2 solely via the growth pattern it induces, not directly. This may safely be assumed on the basis of the discussion of the data in Figure 5.

3.4 Pupal weight

Number of instars As was to be expected from the discussion of larval growth, the number of instars had a pronounced effect on pupal weight. Pupae were heavier, on the average, the higher the number of instars (Table 8).

Temperature Temperature had a positive effect on pupal weight. Table 9 shows this for an experiment under controlled conditions. Moreover, a positive correlation was found (Fig. 6) between mean pupal weight in the rearings in the insectary and the average temperature of July, August, and September together (at de Bilt, see p. 9). This correlation was more pronounced in six-instar individuals than in five-instar ones.

Table 8. Relation between number of larval instars and mean pupal weight in singly reared larvae.

	Mean pupal weight ± standard error in larvae with			
	4 instars	5 instars	6 instars	7 instars
♂♂	89.1 ± 0.95 (n = 240)	122.3 ± 0.35 (n = 1385)	130.9 ± 1.12 (n = 150)	
♀♀	109.7 ± 1.68 (n = 159)	169.5 ± 0.67 (n = 1139)	191.1 ± 1.50 (n = 334)	193.2 ± 10.02 (n = 5)

Pooled data of all experiments. n = number measured.

Fig. 6. Relation between temperature and mean pupal weight of larvae reared singly in an outdoor insectary.

Circles indicate values estimated from the relation between penultimate instar head width and pupal weight, obtained in an experiment in which the larvae were killed in the prepupal stage.

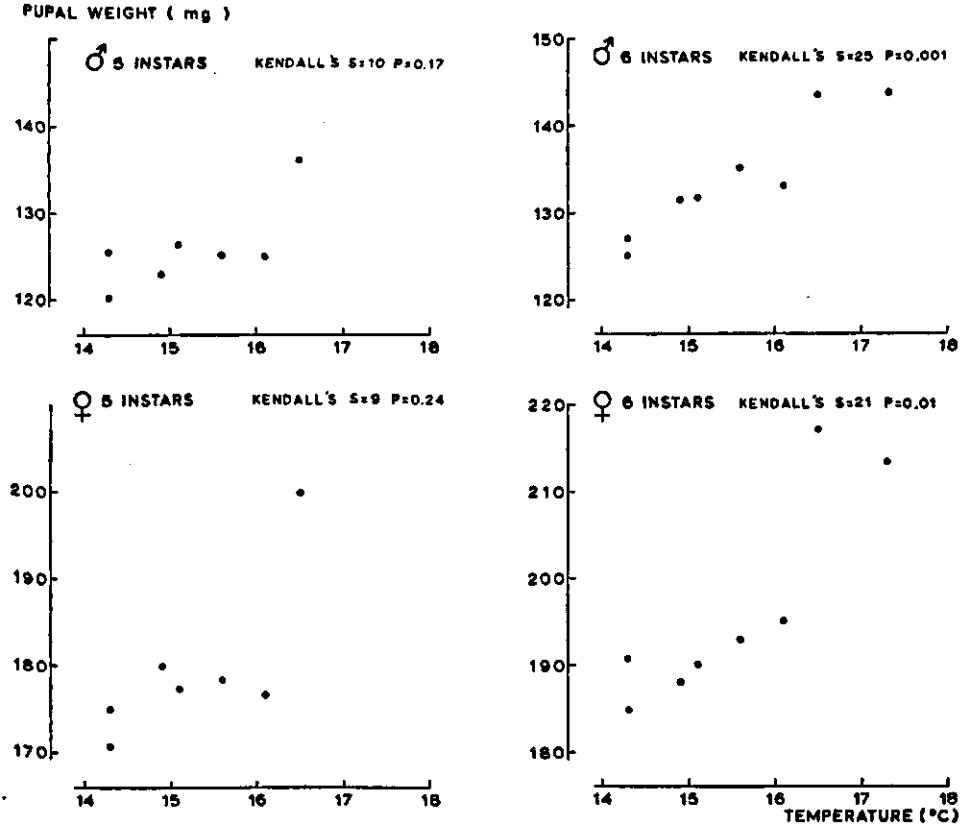


Table 9. Influence of temperature and relative humidity during the larval stage on pupal weight of singly reared larvae.

Temperature (°C)		Relative humidity (%)	Mean weight of pupae (mg)			
day	night		males		females	
			4 instars	5 instars	4 instars	5 instars
24	20	77	97.6 (10)	105.9 (14)	119.6 (8)	152.8 (35)
24	20	94	103.6 (7)	118.5 (23)	121.5 (2)	153.9 (34)
18	10	77	77.6 (28)	80.5 (2)	92.1 (30)	113.6 (5)
18	10	94	83.7 (27)	79 (1)	98.6 (23)	116.3 (3)

For details of conditions, see Table 3. In brackets: number measured.
In all series the prepupae were kept at 15° C.

Table 10. Influence of daylength during the larval stage on pupal weight of singly reared larvae.

Conditions	Range of mean pupal weight (mg) in various experiments	
	5-instar ♂♂	5-instar ♀♀
Natural photoperiod during summer, average temperatures 14.1° to 17.3° C	120–137	171–199
Constant darkness, average temperatures 14° to 22° C	79–125	111–168

Humidity The influence of humidity on pupal weight can only be judged from the effect of two humidities, namely 77% and 94% (Table 9). At the higher humidity, the pupae tended to be slightly heavier.

Daylength Of greater consequence to pupal size were light conditions (see Table 10). Rearing in constant darkness resulted in reduced pupal weight. Since, at natural photoperiods, pupae of larvae that hatched late (from 6 to 29 July) were not consistently lighter than pupae of larvae that hatched early (from 19 June to 6 July), it may be concluded that slight differences in daylength are not reflected in pupal weight.

3.5 Influence of daylength and temperature on the rate of growth

Daylength and temperature had considerable direct influence on the duration of the larval stages (figs. 2 and 4, tables 11 and 12). Although a strict comparison may be made between only some of the series, since most of them were reared in different

years or from different batches of eggs, it seems justified to draw some conclusions. In the laboratory, larval development lasted roughly 100 days under the natural daylength during the period in which the larvae occur in the field (latter half of June, 17 hours light, to October, 11 hours light). Shorter days and constant darkness reduced the duration of the larval stage, and longer days and constant illumination prolonged it (Table 11).

Table 11. The influence of daylength and temperature on the duration of the larval stage of singly reared larvae.

Photoperiod	Temperature (°C)		Mean duration of larval stage (days) in larvae with		
			4 instars	5 instars	6 instars
Constant darkness	controlled day*	18	58	71	
	controlled night*	10			
	controlled**	16	47	56	
	controlled***	16	54		
	controlled day*	24	45	47	
	controlled night*	20			
	controlled**	24		54	
	controlled***	24		49	
Short photoperiod (from 15 hr in L1 to 11 hr at prepupal stage	uncontrolled (insectary) average about	15	61	68	
Natural photoperiod in summer (from 16½–17 hr in L1 to 11 hr at prepupal stage)	uncontrolled (insectary)				
larvae hatched 19/6–22/6	average about	17			114
20/6–30/6		16		99	109
29/6– 1/7		14		105	113
1/7– 4/7		16		94	101
7/7–11/7		15		96	
Long photoperiod (from 14½ hr in L1 to 17 hr in L3, and 11 hr at prepupal stage)	uncontrolled (insectary) average about	15		138	165
larvae hatched 27/4					
Constant illumination	uncontrolled (laboratory and greenhouse) average about	17			286[1]

1. Under these conditions, nearly all larvae died before reaching the prepupal stage in either the 6th, the 7th, or the 8th instar; the figure represents the average age at death.
Series marked with same number of asterisks are from the same experiment.
8–69 observations per mean. Day: 08.00 hr to 20.00 hr; night 20.00 hr to 08.00 hr.

Table 12. Mean duration of stages (in days) of singly reared larvae in relation to number of instars, light conditions and temperature.

Larval stage	a constant darkness 20° to 24° C		b constant darkness 10° to 18° C		c short day av. temp. about 15° C		d normal daylength av. temp. about 14° C		e constant light av. temp. about 17° C
	4 instars	5 instars	4 instars	5 instars	4 instars	5 instars	5 instars	6 instars	
1	5.8	5.9	11.8	14.3	13.1	13.2	14.0	14.5	15.0
2	6.1	6.4	12.1	11.0	8.2	8.5	14.2	14.7	13.2
3	8.5	6.4	12.2	11.6	14.2	11.0	17.8	16.4	23.5
4	24.3	8.1	21.5	12.3	25.8	10.4	24.4	14.2	42.8
5		20.2		22.1		24.6	35.1	19.0	73.2
6								33.9	(118.5)
L1-prepupa	44.7	47.0	57.6	71.3	61.3	67.7	105.5	112.7	(286.2)
No. of obs.	10	69	49	8	13	12	10	11	67

a and b females: for details on rearing conditions see Table 2; the data for the two relative humidities have been pooled here.
c females: reared in the insectary; short day; larvae hatched from 14 to 16 August (see Fig. 2, Series 4).
d females: reared in the insectary; normal daylength; larvae hatched from 29 June to 1 July.
e larvae: reared under constant illumination. Almost all individuals died in L6, L7, or L8, before reaching the prepupal stage; the figures are irrespective of sex and number of instars; those between brackets represent the average number of days from the end of the 5th stage to either the prepupal stage or death.
a and b belong to the same, c, d and e to different experiments.

In Table 12, columns (b), (c), (d), and (e), referring to temperatures within the same range, show the effect of photoperiod on the duration of the larval stages. Especially the later stages, from about L3 onward, were influenced by daylength conditions. L1 and L2 were neither greatly accelerated by constant darkness nor greatly prolonged by constant illumination.

Columns (a) and (b) show the role of temperature: warmth accelerated development considerably in the early stages, and this effect clearly diminished as the larvae grew older. In the last stage, warm conditions may even be deleterious. The upper part of Table 11 shows that the total larval stage at 24° C was not much shorter than at 16°; this was due on the one hand to the extra instar passed through, and on the other to the relatively long duration of the last stage at the supra-optimal temperature.

3.6 Number of larval instars and post-larval development

Morphologically normal pupae were formed after a development through four, five, six, or seven instars. Moths of four, five, and six-instar larvae proved capable of normal reproductive behaviour; seven-instar larvae were not tested in this respect.

The performance of pupae and adults from insectary-reared larvae with five or six instars was compared in some detail (Table 13). No significant differences were found in pupal mortality or the average data of adult emergence. Fecundity was determined by rearing the moths by pairs in a cage, five and six-instar individuals being paired at random. A low percentage of the females remained unfertilized, and no differences between five and six-instar females became apparent in this respect. Besides the number of eggs laid by each fertilized female, the ripe eggs (with a chorion) retained in the ovary and oviducts at death were counted to obtain the total number of eggs produced. In Bupalus, total number of eggs is positively correlated with pupal weight, as is the case in many other insects (see also p. 63). Since pupal weight tended to be higher in six than in five-instar females, the significant difference in the total number of eggs between the two groups agrees with expectation.

The number of ripe eggs retained in the ovary and oviducts at death appeared to be highly variable. The majority of the females retained only a few eggs, but in some cases there was a considerable retention for which no explanation could be found. On the average, six-instar females died with a significantly higher number of eggs in their ovaries than those with five larval instars. Within the two groups, the number of eggs retained was not correlated with pupal weight; therefore, the difference in weight of the pupae between the two categories has no connection with the difference in egg retention.

The number of eggs laid did not differ significantly between the two groups, the greater egg production in six-instar females being offset by a greater retention. No significant difference was found in mean egg weight.

Adult lifespan is slightly longer in six-instar females. Females (of both categories) that performed poorly in laying their eggs showed a tendency to a somewhat prolonged lifespan.

Finally, neither the hatchability of the eggs under different temperature and humidity conditions, nor the resistance of the newly hatched larvae to starvation, was found to be influenced by the number of larval instars of the mother (for experimental procedure, see p. 67).

Table 13. Reproduction and longevity of fertilized female moths in relation to number of larval instars.

	Experiment number				Significance of differences
	1	2	3	4	
Mean number of eggs laid by					
5-instar females	197.0	194.8	204.0	203.4	
					P = 0.63
6-instar females	172.8	167.7	212.7	194.5	
Mean number of eggs retained in ovary by					
5-instar females	22.1	14.2	18.6	36.3	
					P = 0.006
6-instar females	65.3	54.9	43.3	45.8	
Mean total number of eggs of					
5-instar females	219.1	209.0	222.6	239.7	
					P = 0.022
6-instar females	233.1	222.6	256.0	240.2	
Mean adult lifespan (days) of					
5-instar females	12.0	11.4	10.9	11.3	
					P = 0.025
6-instar females	12.9	11.3	12.8	13.1	
Number of individuals tested:					
5-instar females	46	11	26	24	
6-instar females	14	7	21	12	

Data of females from singly reared larvae from 4 experiments in the insectary.
P-values apply to the combined results of the tests in each of the four experiments. The t-test was used for total number of eggs and adult lifespan, and Wilcoxon's test for number of eggs laid and number of eggs retained in the ovary.

4 Discussion

The foregoing implies a high degree of flexibility in the development of *Bupalus* larvae. Under outdoor daylength and temperature conditions during the summer, we found larval development to take three to four months, comprising either five (at an average summer temperature of 15° C) or six instars (at an average summer temperature of 18° C). Both short days during the early stages and constant darkness reduced the larval period to one and a half to two months, with four (moderate temperature) or five (warm conditions) instars. Long days and constant illumination prolonged the larval stage and were attended by a high number of instars, an appreciable number of larvae going through seven instars and some even entering an eighth one. The pattern of growth is also influenced by larval population density (see Part II). Finally, there was a difference between the sexes: females tended to moult once more than males. Under a given set of environmental conditions, variation in the number of moults was rarely greater than one.

Klomp (1966) describes a variability in the number of instars in a field population of *Bupalus* located in the same area as that from which our material was obtained. From an analysis of head widths of larvae he concludes that development comprises either five or six instars, the proportion of the two types being variable from year to year. Some of our observations indicate that a four instar development may occur exceptionally in the field (see p. 13).

Several British authors mention the same variability as that shown by our observations, i.e. from four up to seven instars in rearings (Bevan, Davies & Brown, 1957; Crooke & Bevan, 1957). Crooke (1956) found the four-instar growth type in the field in England.

In the reports on rearing experiments of *Bupalus* larvae under controlled conditions, it is rather astonishing to notice that none of the authors mentions any variability in the number of moults: they all found five larval instars (Hussey, 1957; Oldiges, 1959; Schwenke, 1953). Because of the range of temperatures in their experiments (10° to 25° C) and the probability of an unnatural daylength in thermostat rearings, a variation in the number of instars would be expected.

In the older German literature, development in *Bupalus* larvae is said normally to comprise five instars, and the occurrence of only four instars is mentioned as an exception (Escherich, 1931; Kalandadze, 1927).

It is therefore quite possible that differences in reaction to environmental conditions exist between *Bupalus* larvae from different areas. If this were true, the British literature on the subject would suggest such differences to be rather local. In some

other lepidopterous species differences between individuals from different regions in ability or readiness to develop with a variable number of moults are known or presumed to exist. Jankovic *et al.* (1959) found this in *Lymantria dispar* in Yugoslavia, and Klein (1932) found a variable number of moults in *Pieris brassicae*, in association with differences in environmental conditions in Israel, in contradistinction to the results of David & Gardiner (1962) in England and van der Geest (unpublished report) in the Netherlands.

The comparison of longevity and reproduction of five and six-instar individuals in some detail under laboratory conditions (Table 13) showed no significant advantage of one of these types over the other.

Klomp (1966) mentions larval population density as the cause of the variability of the number of instars in the field. The importance of temperature, however, can also be readily demonstrated from Klomp's data by an analysis of the regression of the percentage of six-instar larvae in the various years on larval density and temperature. Temperature was taken as the average from April through August at De Bilt (see p. 9), the spring months having been included in view of the influence of the hatching date of the larvae on their growth pattern; hatching is earlier, the earlier the adults emerge, i.e. the higher the temperature during the spring. Seventy per cent of the variation in percentage of six-instar individuals can be attributed to regression, 60% of this is due to temperature, the remaining 40% being attributable to larval density.

In contrast to Hussey (1957), Oldiges (1959), and Schwenke (1953), we found a positive correlation between temperature during the larval stage and pupal weight in the same range of temperatures as in their experiments. In addition, our pupae were considerably heavier, but this is probably due to differences in rearing density. As to the effect of a decrease in the optimum temperature in the last larval stage, our results agree with those of the authors mentioned above. The unfavourable effects of high temperature and long photoperiod in the later stages are not surprising in view of the life-cycle of *Bupalus* in the field. In Templin's (1960) experiments, a daylength of 17 hours even resulted in mortality of 100% in L3 and L4.

Several factors influencing the number of instars in insects are known. Races with different, and genetically determined, numbers of instars occur in some species. In many cases the number of instars is related to sex, and it is the female that tends to develop through an additional instar (Eidmann, 1962; Schwerdtfeger, 1963; Wigglesworth, 1965). Parental age may influence the number of moults of the progeny (Ludwig & Fiore, 1960), or, as is the case in some species of locust, parental density determines the size of the larvae at hatching, which is, in turn, determinative for the number of instars, small larvae developing with an additional moult (Albrecht, 1955).

As to external factors, temperature, humidity, quantity and quality of food, crowding, mutilation, infection with *Nosema*, and experimental interference with the hormonal system are mentioned as influencing the number of moults by Eidmann

(1962), Fisher & Sanborn (1964; infection with *Nosema*), Rummel (1963; mutilation: amputation of appendages), Schwerdtfeger (1963) and Wigglesworth (1965). In several cases, the number of instars varies with geographical latitude or season, the actual causative factor being unknown (e.g. Pagès & Almanzov, 1964; Peterson & Haeussler, 1928).

To the best of my knowledge, there is no mention in the literature of a direct effect of the photoperiod on the number of instars in insects. Bogavac (1959) found differences in the number of moults between the first and the second generation of *Hyphantria cunea*, which she presumes to be due to an indirect influence of photoperiod, via the condition of the food plant; she found no direct effect of light conditions. In some Crustacea, it has been shown that light conditions can influence the occurrence of moults (Bliss, 1954; Stephens, 1955).

In *Bupalus*, I have obtained no indication of an influence of external factors other than daylength, temperature, and degree of 'crowding'. That there is little, if any, effect of the condition of the food plant is borne out by the fact that the various growth types were obtained during all seasons by rearing under the appropriate conditions of daylength and temperature, despite the supposedly considerable differences in composition of the pine needles at different times of the year. Our results also failed to provide any evidence suggesting a genetic determination of the precise number of moults. However, individual differences in aptitude to react with an additional instar to a given set of environmental conditions are undoubtedly genetically fixed.

At first sight, the literature concerning the effect of temperature on the number of instars seems to be rather contradictory. Increasing temperature provokes, as in *Bupalus*, more moults in some species (e.g. *Ephestia kuhniella*, v. Gierke, 1932; *Dermestes lardarius*, Kreyenberg, 1929; *Ptychopoda seriata*, Langen, 1938; *Sphodromantis viridis*, Przibram, 1909) but causes fewer moults in others (e.g. *Pieris brassicae*, Klein, 1932; *Malacosoma neustria*, Laux, 1962; *Melanoplus mexicanus*, Parker, 1930).

In view of these seemingly conflicting results in different species, the papers by Blake (1959), Herfs (1936), and Mayer (1940) are of special interest. These authors found the smallest number of moults at a specific, medium-range temperature and an increasing number at higher and at lower temperatures, in individuals of the same species (*viz.*, *Anthrenus verbasci*, *A. fasciatus*, and *Lymantria monacha*, respectively). It is known that deviation from the optimum in other factors, such as the quality or quantity of food, can bring about a spectacular rise in the number of instars in association with retarded development (e.g. Herfs, 1936 and Titschack, 1926, in insects of stored products; and Gaines & Campbell, 1935, Kurir, 1952, Long, 1953, and Peterson & Haeussler, 1928, in phytophagous species). Hence, in the case of temperature, retardation of development could conceivably be the cause of the higher number of instars at the lower values. Since growth is accelerated rather than retarded in at least a certain range of temperatures above the medium values in *A. verbasci*, *A. fasciatus*, and *L. monacha* (references cited above), this cannot apply to the increasing number of moults at the higher temperatures.

Therefore, it is suggested that temperature may act directly as well as indirectly on the number of instars; the direct influence leading to an increasing number of moults with rising temperature, and the indirect influence to an increasing number of moults with decreasing rate of development. Below a certain specific medium temperature, the indirect effect predominates, and above it the direct one.

In my experiments in which temperature was the only variable factor, I consistently obtained a greater number of moults and a higher rate of development at the higher temperatures (tables 3, 5, 11, 12), which means that I found only the direct effect of temperature. Furthermore, the wide range of the duration of the larval stage in the five-instar growth type shows that the growth rate can hardly be of great influence on the number of instars in *Bupalus* (Fig. 4).

Similarly the question arises whether photoperiod acts directly or indirectly on the pattern of growth. Since long photoperiods considerably diminish the growth rate from about the middle of L3, the supposition of an indirect action via the growth rate might seem plausible. But a case study of the individuals in the long-day series shown in Table 4 revealed no correlation between rate of growth and number of instars, which supports the conclusion that the effect of daylength is a direct one.

It is important to stress once again the early determination of the number of moults in *Bupalus*. Head capsule measurements in several other Lepidoptera with a variable number of instars lead to the same conclusion (Gaines & Campbell, 1935; Iwao, 1962; Langen, 1938; Long, 1953; Peterson & Haeussler, 1928).

The question arises whether the sensitivity to environmental factors determining the pattern of growth is restricted to a brief developmental stage. We have seen (Section 3.3) that although this is probably not the case, the final part of the sensitive period is rather critical, owing to the impossibility of any further change in the induced pattern. From the evidence presented, the following picture can be drawn. The induction of the number of instars by external factors begins at some moment in the embryonic stage. At the end of L2, it has been determined in the majority of the larvae whether the ultimate number of instars will be four or more; at the end of L3, whether it will be five or more; and so on (Table 5; Fig. 3).

Although the endocrine processes operating at moulting and metamorphosis are well known, the hypotheses put forward to explain the physiological mechanism responsible for regulation of the number of instars and the amount of growth in each instar are controversial. Novák (1966) holds the view that the relative deficiency of the juvenile hormone provoking metamorphosis (or pupation) arises from the progressive decrease in the ratio of the surface area of the corpora allata and the body volume with increasing body size, as a result of which the amount of juvenile hormone that can be released in the last larval instar is too small for the next ecdysis to be larval. According to this view, the functioning of the corpus allatum represents the mechanism regulating the number of instars.

On the other hand, from the results of transplantation experiments with corpora allata in *Rhodnius*, Wigglesworth (1964) concludes that '. . . it is not the corpus

allatum which counts the instars'; instead, a nervous stimulus from the brain would inhibit the secretion of juvenile hormone during the last larval instar. A nervous inhibition of the corpora allata has indeed been demonstrated for the adults of several insect species (Engelmann, 1965; de Wilde, 1965).

Since additional instars have been provoked by implantation of corpora allata and precocious metamorphosis has been shown to result from allatectomy in several insects (Wigglesworth, 1964), a change in corpus allatum functioning might conceivably be involved in the variability of the number of instars in *Bupalus;* but the situation is clearly more complicated than the mere addition or omission of an instar. What is involved is not merely a *counting* of instars, but rather a regulation of development through *different programs;* with a higher number of instars the smaller the amount of growth per instar and the shorter the intermoult periods, from a certain instar onwards (Fig. 3; Table 12). These effects have some resemblance to the effects of experimental interference with the ventral gland. In Staal's (1961) experiments, the implantation of ventral glands in *Locusta* larvae in the first or early in the second instar led to the enhancement of wing development, prolongation of the second stage, loss of one instar before metamorphosis, and a reduction in the size of the adults. When the implantation was performed at a later stage, these effects did not occur.

Halbwachs et al. (1957) and Joly (1962) also reported an effect of ventral gland implantation on the rate of development in *Locusta*, albeit an effect more or less opposite to that found by Staal: after implantation of ventral glands in the beginning of L4, moulting was accelerated and the intermoult period shortened, and there was a reduction in the length of the femora of the metathorax and of the pterothecae.

It therefore seems likely that the primary change leading to the different growth patterns in *Bupalus* consists of a modification of the amount of growth per instar induced by a change in the functioning of the prothoracic gland (which is homologous to the ventral gland in the above-mentioned insects) and that, as a secondary effect, a change in the number of instars occurs. In this respect, we may also consider homeostatic responses such as those mentioned by Wigglesworth (1964) for *Rhodnius prolixus*. If growth in *Bupalus* is controlled by homeostatic mechanisms, a difference in growth pattern could only be caused by a change in the 'thermostat'. And since the prothoracic glands are subordinated to the intercerebral neurosecretory cells, it is highly probable that the primary induction of the changes in growth pattern described above for *Bupalus* takes place in the brain, where the centre of neuro-endocrine integration is located (Scharrer, 1959). In this respect, the photoperiodic effect on the number of instars is of interest, because it has been found that photoperiodic induction takes place in the brain (Lees, 1964; Williams & Adkisson, 1964).

However, before the observed influence of environmental factors on the developmental pattern can be interpreted in terms of a change in the mechanism coordinating development, experimental interference with the endocrine system will be necessary. This research could be performed advantageously in a species such as *Bupalus piniarius*, which shows a natural variability of the growth pattern under the influence of environmental conditions.

Part II. The effect of larval density

1 Introduction

Having considered the influence of abiotic factors on growth of *Bupalus piniarius* in Part I, we now come to the effect of population density, already introduced briefly (p. 1).

Growth in *Bupalus* is negatively correlated with larval density (Klomp, 1958a, 1966). What makes this relation puzzling is, that the population concerned is very thin relative to the abundant supply of food, and that pine loopers are sluggish. Competition for food can therefore be excluded as its cause and mutual interference, at first sight, seems unprobable. But the relation between density and growth needs not necessarily be causal. It could conceivably arise from the dependence of both density and growth on a third factor. Klomp (1958a) has argued from his field data against such dependence on a third factor. Another approach is to attempt to reproduce the effect of density in experiments. Some laboratory results of this sort, supporting the assumption of a causal relationship, have already been published (Klomp, 1966; Klomp & Gruys, 1965). These data are extended in Chapter 2 to a description of the experimental effect of larval density on the individual. Results from laboratory experiments provide only a meagre basis for the conclusion that growth is causally related to population density. Field experiments attempt to bridge the gap between jam jars and nature.

The impact of larval density on growth in the field is compared with the influence of abiotic factors.

In Chapter 3, the mechanism of the density effect is studied.

Finally, in the comparing of the effect of density in *Bupalus* with similar phenomena in other species of insect, its possible function is examined (Chapter 4).

In the following, the adjectives 'single' and 'solitary' are used interchangeably in connection with larva, individual, rearing, series, group, category, to indicate that the larvae in question were reared singly in separate vials. Similarly, the adjectives 'crowded', 'grouped', and 'aggregated' mean that two or more larvae were reared together in one vial, throughout their larval life unless otherwise mentioned. 'Density' covers population density in the field as well as crowding conditions in rearing experiments. It should be stressed that in aggregated and in single rearings, food was always present to excess.

Only the effects of larval aggregation were studied; hence, 'density', as used in the term density effect, refers to conditions during the larval stage.

Materials and methods used in this study have been described in Part I.

2 Experimental analysis of the effect of larval density

Differences in growth are most easily determined on the basis of pupal size, so I shall first consider this quantity, as a parameter of the influence of density in rearing and field experiments, and then go into the influence of larval density on the various instars in more detail.

2.1 Comparison of the effect of larval density in experiments and in the field

The influence of larval density on pupal size can be easily demonstrated in simple rearing experiments; Figure 7 and Table 14 give an example. They show that, when two larvae were reared in one jar, mean pupal weight was reduced by 20% in the females and by 15% in the males as compared to singly grown caterpillars. A further increase in density in these experiments (five larvae per jar) caused only a slight further reduction in size, reaching the level of significance only for the females. Total reduction in weight, with density increasing from one to five larvae per jar, amounted to approximately 24% in the females. As in all comparable experiments, size was less decreased by density in the males than in the females, and in the males the total reduction already resulted from the first step of density increase.[1]

In another series of the same experiment, the larvae were reared in pairs in five-litre containers provided with a proportionally greater amount of pine twigs than in the control series in 0.37-litre jars. This series was added to ascertain whether an intermediate effect, comparable to the gradual reduction in size with increasing density in the field, could be obtained in this way. This appeared not to be the case.

Since a further increase in container size was impracticable in the laboratory, three experiments on this point were conducted in the field.

a. Larvae were reared in aggregation on 12-year old Scots pines, on branches of different size covered with cheesecloth bags.

1. Pupae reared in the insectary are slightly larger than those from the field, but the percentage reduction in size due to experimental crowding and the size reduction due to the highest larval density encountered in a natural population, are approximately equal (compare Table 14 with the field data in Table 16, columns 9–12).

Fig. 7. Influence of larval density on weight of pupae (in mg) in insectary rearings.

$\bar{x}$ = mean weight, n = number of observations, s = standard deviation.
Significance of differences (t-test) between 1 and 2 larvae per jar: $P < 0.001$ in both males and females, between 2 and 5 larvae per jar: $P = 0.6$ in males and 0.07 in females.

In all series the larvae passed through five or six instars (for details see Part I). The difference between these two groups has been ignored because in the series with two or more larvae per jar the number of instars could be ascertained only for some of the pupae. In the crowded series, the six-instar growth pattern was less frequently represented than in the single group, but this could at best account for a small part of the size reduction associated with increasing density, as is obvious from a comparison of the effect of density with the difference in weight between five and six-instar individuals. Average pupal weight of singly reared individuals: males, five instars = 137.1 mg, six instars = 143.3 mg; females, five instars = 199.1 mg, six instars = 216.7 mg.

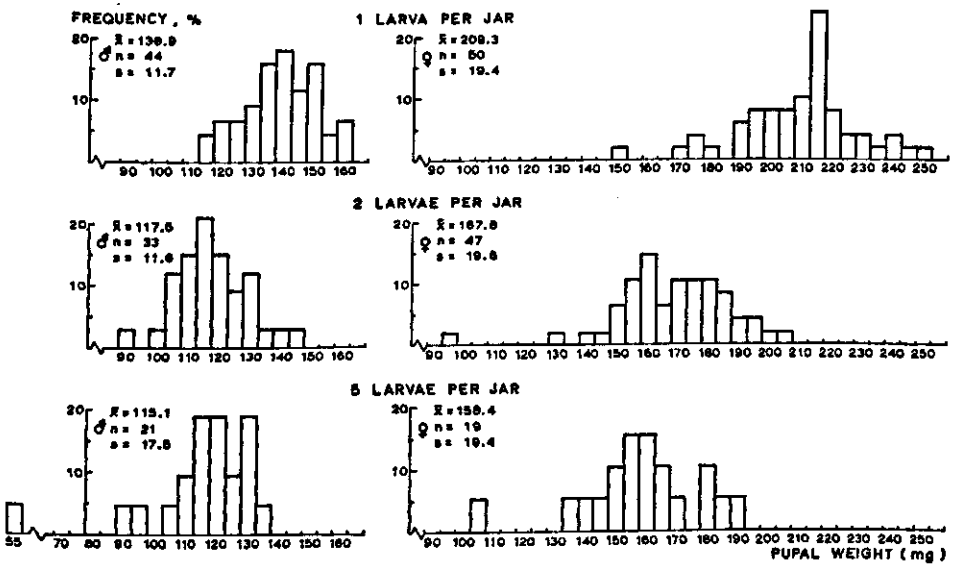


Table 14. Mean diameter of pupae (mm) in the experiment shown in Figure 7.

Density per jar	Males	Females
1 larva	4.58 ± 0.02 (n = 44)	5.38 ± 0.03 (n = 50)
2 larvae	4.34 ± 0.03 (n = 33)	4.98 ± 0.03 (n = 47)
5 larvae	4.28 ± 0.05 (n = 21)	4.86 ± 0.06 (n = 19)

Standard error and number of observations (in brackets) are given.
Significance of differences (t-test): between 1 and 2 larvae per jar, $P < 0.001$ in males and females; between 2 and 5 larvae per jar, $P = 0.3$ in males and 0.06 in females.

In the evaluation of the results, pupae from bags containing only one *Bupalus* and no dead specimens were treated as a separate group (Table 15, first line). The means of the females in the large and medium-sized bags were clearly intermediate between the means of those in the small bags and in the 'singly' reared group. For the males, gradual changes in the effect were absent, the only obvious difference being between the 'single' group and all other series.

b. In a similar experiment conducted simultaneously in the same plantation, bags of medium size were provided with different numbers of eggs about to hatch. The bags were taken from the trees in October, and subsequent treatment of the larvae was similar to that in the experiment under *a.* The number of larvae present in the autumn varied per bag between 2 and 41. Again, for the females there was a clear distinction in size between high and lower density. For the males, no gradation in size was found.

c. Different larval densities were set up on several groups of five trees in a 35–year–old Scots pine forest in which pupal density in the preceding winter had been low (Table 16).

Pupal diameter (Table 16, columns 7 and 8) showed a highly significant decreasing

Table 15. Density effect in a field experiment with larvae caged on branches of different size.

Number of larvae per bag (in autumn)	Content of bags (litre)	Mean number of larvae per young shoot	Mean pupal weight (mg)		Mean pupal diameter (mm)	
			males	females	males	females
1	all sizes combined		111.3 ± 6.1 (n = 4)	151.2 ± 4.1 (n = 11)	4.41 ± 0.10 (n = 3)	4.90 ± 0.05 (n = 11)
2 – 3	50	0.048	101.4 ± 4.6 (n = 7)	141.2 ± 6.1 (n = 5)	4.15 ± 0.06 (n = 7)	4.74 ± 0.08 (n = 5)
2 – 3	30	0.140	100.1 ± 4.6 (n = 7)	144.1 ± 5.2 (n = 7)	4.21 ± 0.06 (n = 7)	4.78 ± 0.07 (n = 7)
2 – 3	3	0.200	98.6 ± 4.0 (n = 9)	129.8 ± 3.1 (n = 20)	4.22 ± 0.06 (n = 8)	4.63 ± 0.04 (n = 19)

Mean ± standard error, and number of observations are given.
Significance of a downward trend in pupal weight: females $P < 0.001$, males $P = 0.16$. In males, the significance of the differences in pupal weight between the series with one larva per bag, and all other series, is $P = 0.09$.
Cylindrical bags were fixed on the trees before wild moths started laying eggs. Small bags covered one bunch of young shoots at the end of a branch, medium sized ones covered the yearling end of a branch with several bunches of young shoots, and large ones covered the one and two-year old part of a branch with the attached young shoots. In each bag three eggs at the point of hatching were attached to the shoots as far apart as possible. At the beginning of October the pupae were collected. Larvae not yet pupated were reared singly in the laboratory until pupation.

Table 16. Effect of larval density on pupal size in a field experiment, compared with Klomp's results from a natural population.

1	2	3	4	5	6	7	8	9	10	11		12	
Number of groups × number of trees per group	Mean number of eggs introduced per tree	Relative larval density in Aug.	Sept.	Mean number of larvae per young shoot in September	Mean number of pupae per m² in winter	Mean diameter of ♂ pupae ± standard error (mm)	Mean diameter of ♀ pupae ± standard error (mm)	Data from natural population (Klomp, 1966) year	mean number of larvae per young shoot Aug. – Sept.	mean diameter (mm) ♂ pupae		♀ pupae	
1 × 5	545	8.5	6.0	(0.030)	4.8	4.49 ± 0.03 (n = 26)	4.97 ± 0.04 (n = 22)	1955 1950 1954 1956	0.028 0.027 0.025 0.025	4.30 4.31 4.30 4.27	4.30	4.77 4.91 4.83 4.69	4.80
2 × 5	296	2.7	2.4	(0.012)	1.0	4.40 ± 0.03 (n = 26)	5.08 ± 0.04 (n = 29)	1959 1953	0.011 0.010	4.39 4.45	4.42	4.94 5.01	4.98
4 × 5	65	1.2	1.2	(0.006)	0.7	4.48 ± 0.03 (n = 29)	5.15 ± 0.04 (n = 21)	1958	0.006	4.49		5.22	
natural population[1]	0	1.0	1.0	0.005	0.3	4.45 ± 0.02 (n = 105)	5.12 ± 0.02 (n = 91)	1952 1957	0.004 0.003	4.37 4.40	4.39	5.03 5.13	5.08

1. On the trees surrounding the experimental groups.

Data in brackets in Column 5: calculated from density of natural population (0.005) and data in Column 4. n = number measured.

Significance of a decreasing regression of pupal diameter on larval density: females $P = 0.003$ (regression coefficient $= -0.322$), males not tested.

Technical details. Different densities were obtained by introducing laboratory reared eggs in the trees. Needles with eggs on the verge of hatching were attached to the shoots with pins. They were distributed as evenly as possible over the crown. On days with low wind velocity, in August and September, relative larval densities were assessed from the number of faecal pellets dropped on small tables under the trees, their tops fitted with grease-proof paper lightly smeared with tanglefoot for adhesion. The data of several days were pooled (columns 3 and 4). Faeces of larvae of other families can be distinguished from those of *Bupalus* but those from other geometrids cannot. The latter fact gave only slight bias, since *Bupalus* larvae were about ten times more numerous than the other geometrid larvae together. For further details on this method see Tinbergen (1960).

In the trees surrounding the plots, larval density was assessed in September from a sample of branches on which the larvae were counted; Klomp's method (1966) was used, to make the results comparable to his data (Column 5).

trend with increasing larval density for the females. For the males, the trend was not clear, the value in the high density plot being aberrant. In view of the lower reactivity of males to density this is not very surprising.

In the relevant data from Klomp's study (Table 16, columns 9–12) the reduction in size of female pupae is greater over a comparable range of larval densities, than it is in the present experiment. Several explanations for this divergence can be suggested, the most plausible being the difference between the two forests concerned. The forest used for my experiment had wider spacing, the number of trees per unit area being about two thirds of that in Klomp's study area, which may mean that for my plantation the unit of foliage used in the expression of density, i.e. one young shoot, represents a larger amount of pine needles. This relatively large amount of foliage could modify the density effect because it will reduce the chance of encounters between larvae (see the mechanism of the density effect, Chapter 3). With a greater amount of foliage more food is, of course, also present; but this is immaterial, since food was available to excess in both cases. The comparability of the density measurements was not studied. Nevertheless, it seems worth mentioning that the nature of the trees may well be an important factor in the relationship between density and growth.

The results from this section can be summarized as follows. There is an obvious agreement between the experimental results obtained in both the laboratory and the field, and the data from Klomp's field population. Higher density of larvae results in smaller size; and this effect is more pronounced and shows a clearer gradation in the females than in the males. Even two or three larvae in a relatively large space (5 to 50-litre volume) or, in terms of field conditions, larvae at a low density, influence each other's growth. The maximum effect is already reached at a rather low density.

2.2 Variability of the effect of aggregation

The effect of density on pupal size in insectary rearings in the different years of the investigation was highly significant in each case, but rather considerable differences between experiments with identical density conditions were found in the absolute and the relative effect. The reduction in pupal weight in seven series with five larvae per jar, expressed as a percentage of the respective solitary controls, ranged from 12 to 24 % in females and from 9 to 17% in males. The cause of this variation remained unknown.

Under laboratory conditions deviating to some extent from natural circumstances but still permitting the completion of larval development, a clear effect of density was still found, especially for the females. The density effect was observed in larvae with four as well as those with five or six instars. Larvae of both sexes had, on the average, the same influence upon members of their own as upon those of the opposite sex.

A point of interest is whether, at the same degree of crowding, all larvae are equally affected – apart, of course, from the differences existing between the sexes.

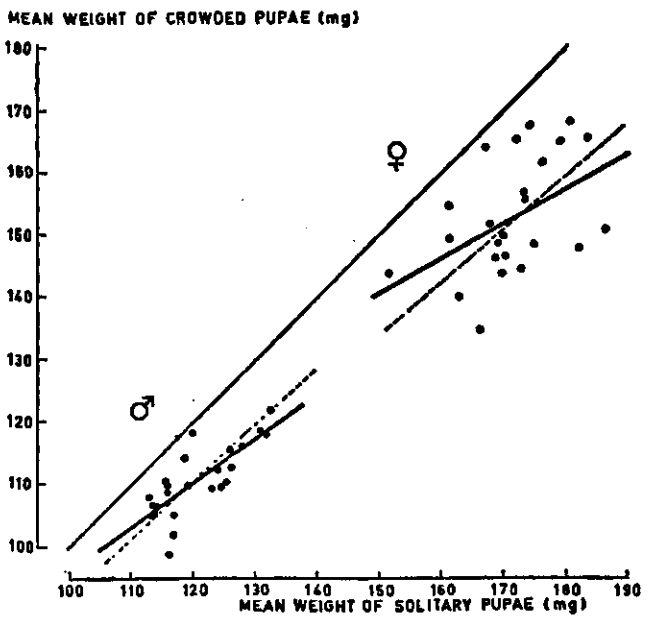
The fact that the standard deviations of the frequency distributions in Figure 7 within sexes do not seem to differ between the series[1] does not necessarily prove this to be the case. Because of its importance to the question of the function of the density effect, this point was investigated in a separate experiment.

Since it was supposed that differences in susceptibility to the effect of density might be genetically determined, the eggs for this experiment were taken from separately reared pairs of moths. From the eggs of each of 24 pairs of adults, 24 randomly chosen larvae were reared in the insectary singly, and 24 in crowds of six larvae per jar, for comparison of the effect of density between the progenies. In this way, crowded larvae were subjected solely to the influence of their brothers and sisters.[2]

The results are represented in Figure 8, in which for each progeny the mean pupal weight of the crowded larvae is plotted against mean pupal weight of the solitary individuals. In the absence of an effect of density, the points would lie scattered about

Fig. 8. Regression of mean pupal weight of crowded individuals on mean pupal weight of solitary individuals in 24 progenies.

Heavy lines: sample regressions: ♂♂ $y = 0.721\,x + 23.617$, ♀♀ $y = 0.563\,x + 56.252$
Dashed lines: ♂♂ $y = 0.919\,x$, ♀♀ $y = 0.880\,x$
Thin lines: $y = x$, in case of no effect of density.



1. Similar results were found in most of the other experiments of this kind.
2. A design permitting analysis of the effect of larvae from different progenies upon each other would have required the marking of the individual larvae in the crowded series, which was impracticable.

a line passing through the origin and making an angle of 45° with the abscissa. The actual situation of the points relative to this line plainly shows the effect of density. The differences in density effect between the various progenies seem to be considerable.

To test the significance of these differences, the data were subjected to an analysis of variance (Table 17), in which the interaction component is the interesting one in view of the purpose of the experiment. Since the reduction in weight caused by density was assumed to be a certain percentage of solitary weight rather than a certain absolute value, the analysis was conducted on the logarithms of pupal weight, so that the interaction sum of squares would represent the differences between the quotients of the crowded and the solitary weights of the various progenies. The subsequent regression analysis (see below) showed this procedure to be appropriate.

In the females, the interaction was significant, indicating that the percentage weight reduction differed between progenies. In the males, the interaction did not reach significance.

Table 17. Analysis of variance of the logarithms of pupal weight from an experiment with larvae of 24 progenies reared singly and in crowds.

Source of variation	Males			Females		
	degrees of freedom	sum of squares	mean square	degrees of freedom	sum of squares	mean square
density	1			1		
progenies	23			23		
interaction	23	0.018463	0.000803	23	0.054536	0.002371
individuals	381	0.443813	0.001165	353	0.520732	0.001475
	Interaction: $F = 0.69$, not significant			Interaction: $F = 1.60$; $F_{0.05}(df.23;353) = 1.56$ $F_{0.01}(df.23;353) = 1.87$		

Table 18. Analysis of variance of pupal weight of singly reared individuals in 24 progenies.

Source of variation	Males			Females		
	degrees of freedom	sum of squares	mean square	degrees of freedom	sum of squares	mean square
total	233	27547		198	46291	
progenies	23	9660	420.0	23	9285	403.7
individuals in progenies	210	17887	85.1	175	37006	211.5
	$F = 4.93$ $F_{0.001}$ (df.23;210) = 2.40			$F = 1.91$ $F_{0.01}$ (df.23;175) = 1.92		

A separate analysis of the solitary individuals (Table 18) showed significant differences in size between the various progenies; these differences may safely be attributed to genetical factors.

The regression coefficients (males, $b = 0.721$; females, $b = 0.563$) were compared with certain hypothetical values (see Fig. 8).

The first of these values was $\beta = 0$. In combination with the difference in size between crowded and solitary pupae, this would mean that, on the average, crowding reduced weight to a certain fixed value in all progenies. Clearly, this does not apply, since the sample values differed significantly from the hypothetical value (males, $P < 0.001$; females, $P = 0.02$).

Secondly, the sample regression coefficients were compared with $\beta = 1$, which would mean that, on the average, the reduction in weight due to crowding was by the same absolute quantity in all progenies. Again, a significant deviation from the hypothetical value was found (males, $P = 0.03$; females, $P = 0.07$).

Thirdly, it might be supposed that crowding caused weight to be reduced, on the average, by a certain fixed percentage. The regression coefficients would then be expected to be $\beta = 0.919$ in males and $\beta = 0.885$ in females (viz. the over-all mean of crowded pupae divided by the over-all mean of solitary pupae; dashed line in Fig. 8). The deviations of the sample values from the hypothetical again seemed rather large, and the fact that they were in the same direction for both sexes provides an indication for a greater percentage reduction in progenies consisting of big individuals than in those of small ones (males, $P = 0.10$; females, $P = 0.17$). The considerable scatter of the points around the regression line suggests that, besides genotypical size, other unknown factors were important in determining the reduction in growth by density.

2.3 Effect of aggregation on larvae

Mortality Table 19 shows the data pertaining to the relation between density in rearing experiments and mortality in the larval stage, which suggest a slight increase of the incidence of deaths with increasing density. Although the differences in mortality between densities were significant for only four experiments, higher mortality was associated with higher density in 12 out of 16 cases, and a sign-test showed the probability of such a result, under the hypothesis of no difference, to be $P = 0.10$. However, I suppose that the difference is not real, and the column 'mortality from accidents' supports this supposition. The term accidents refers to individuals known to have died from rough handling (these cases were not, of course, included in the percentage 'natural' mortality). Accidents were more frequent in the crowded series, and it is conceivable that unnoticed accidents that caused the death of an individual at a later date, also occurred more frequently in the crowded series, thus giving a false impression of a somewhat higher natural mortality. The crowded larvae of Experiment 11 (three individuals per small piece of shoot) may form an exception to this supposition. They were observed to leave the twigs very frequently, and to

Table 19. Mortality of larvae in rearing experiments in relation to density.

Experiment	Mortality (%) from natural causes					Mortality (%) from accidents				Rearing conditions[2]
	larvae per jar					larvae per jar				
	1	2	5	3[1]	P(H_0)	1	2	5	3[1]	
1	13	17	22		>0.50	2	6	5		July-Oct.; insectary; 15°–17°C); 16–17 hr (L1) → 11 hr (prepupa).
2	10	15			>0.50	2	9			
3	15		26		0.04	12		20		
4	21		25		>0.50	3		2		
5	17		24		0.16	4		17		
6	15		13		>0.75	5		7		
7	9		15		0.35	2		10		
8	22		19		>0.50	3		10		
9	18		29		0.02	3		9		
10	15		21		0.02	3		9		
11	15			35	<0.005	7			11	
12	29	34			>0.50	1	10			July–Aug.; thermostat cabinet; 16° C; constant darkness.
13	36	38			>0.75	0	6			July–Aug.; thermostat cabinet; 24° C; constant darkness.
14	29	27			>0.75	6	6			May–Sept.; greenhouse; 19° C; 17 hr (L1–4); 17 → 12 hr (L5-prepupa).
15	17	10			0.40	2	15			Aug.–Oct.; insectary; 15° C; 15 hr (L1) → 11 hr (prepupa).
16	30	39			0.40	2	5			Jan.–March; greenhouse; 17° C; 17 hr (L1) → 11 hr (prepupa).

1. Larvae of this series and of its solitary control were reared on 3–5 cm long shoot pieces (in the other experiments 15 cm shoots were used). This series is considered to represent the highest degree of crowding.

2. Descriptions refer, in the given order, to: time of year of the experiment; place; (average) temperature; photoperiod during the different stages (arrows indicate a gradual or stepwise decrease).

Number of larvae per series varied from 50 to 650.

P(H_0): probability of the result under the hypothesis of no difference between series (χ^2-test).

produce relatively large amounts of diffuse silk webbing on the walls of the jars. In this case, enhancement of mortality by a very high degree of mutual disturbance is plausible.

The causes of 'natural' mortality in the rearings were not investigated in detail. In L1 and in the prepupal stage, mortality was clearly higher than during the rest of the larval stage. Solitary and crowded series behaved similarly in this respect. In Experiment 1, some of the larvae died from a virus infection in the midgut (2.5%, 8.0%, and 5.3% of the individuals in series with 1, 2, and 5 larvae per jar, respecti-

vely), the main manifestation of which was a sharply bounded, yellow ring around one or two abdominal segments. Clear indications of this disease were not found in any of the other experiments, and it proved impossible to induce its occurrence in experiments with extra-high rearing density.

Size Aggregated larvae are smaller than singly reared individuals at comparable moments from the second instar onwards, as shown by head width measurements in several experiments. Moreover, the duration of the larval stage is longer in aggregation. These differences, together with food intake and faeces production, were evaluated in greater detail in two experiments.

The first of these experiments (Table 20), which was conducted in the insectary during the summer, consisted of one series in which the individuals were reared singly and another series in which the larvae were kept in groups of three, in both cases from immediately after hatching up to the prepupal stage. The larvae were examined daily and were weighed at each moult when, as Figure 9 shows, food intake and defaecation have stopped and the weight remains relatively constant.[1]

The results are given in detail in Table 20, and in a summarized form in Figure 11.

In L1, density did not affect growth. The same result was obtained in some other experiments (see Section 3.5). In L2 to L4, the difference between the two series was considerable, aggregated larvae developing more slowly and lagging behind in weight as compared to solitary ones. The amounts of food consumed and of faeces produced during these stages, however, did not differ essentially between the two series. Curiously enough, this situation was altered in the last stage, which was shorter in the aggregated larvae. Food consumption and faeces production were lower in the crowded larvae in this stage. The shorter duration of L5 in crowded larvae could conceivably be an indirect result of aggregation: due to their relatively long earlier stages moulting to L5 occurred at a later date and L5 was passed during slightly shorter days than in the single series, which could have speeded up growth (see Part I Section 3.5). However, the data in Table 21 plainly show the fallacy of this explanation: when moulting to L5 on the same date, aggregated larvae still passed through their last instar more rapidly than single specimens.

Aggregation exerted its greatest effect in L3 (Table 20 and Fig. 11). Increase in weight, food intake, and faeces production on the one hand and duration of the stages on the other can be expressed in the same figure if the first mentioned quanti-

1. In Fig. 9, food intake, weight, and age are expressed on a relative scale to permit the representation of the data of different larvae in the same graph. Food intake during intervals amounting to one fifth of the duration of a stage, is expressed as a percentage of total food intake during that stage. Ages at weighing are expressed as a percentage of the duration of a stage, and weight as a percentage of total weight increase during that stage.
The points at which the solid lines (under the abscissae) change into dashed lines give the average relative ages at the beginning of moulting (L3 and L4) or prepupation (L5). The duration of the prepupal stage was not determined.

Table 20. Growth, food intake, and faeces production in solitary and crowded larvae (3 per jar)

	1st instar ♂ + ♀		2nd instar ♂ + ♀		3rd instar ♂		3rd instar ♀	
	solitary	crowded	solitary	crowded	solitary	crowded	solitary	crowded
1 Duration, days	*12.0* ±0.2	*12.0*	*12.6* ±0.1	*14.5*	*10.8* ±0.3	14.1	*12.2* ±0.5	15.9
2 Weight at end of stage, mg	*1.55* ±0.06	*1.63* ±0.04	*5.39* ±0.09	*4.84* ±0.07	*14.18* ±0.26	11.42	*15.29* ±0.34	12.34
3 Weight increase per stage, mg	1.35	1.43	3.84	3.21	8.79	6.58	9.90	7.50
4 Weight increase per day, mg (*3*:*1*)	0.11	0.12	0.31	0.22	0.81	0.47	0.81	0.47
5 Food intake per stage, cm	*8.01* ±0.22	*7.60*	*14.18* ±0.50	*14.00*	*20.74* ±0.76	22.20	*23.27* ±0.80	24.85
6 Food intake per day, cm (*5*:*1*)	0.67	0.63	1.13	0.96	1.92	1.57	1.90	1.57
7 Dry faeces per stage, mg	*1.35*	*1.41*	*4.37*	*4.39*	*12.56* ±0.32	13.01	*14.71* ±0.35	15.61
8 Dry faeces per day, mg (*7*:*1*)	0.113	0.118	0.347	0.303	1.16	0.92	1.21	0.98
9 Weight incr. per cm food, mg (*3*:*5*)	0.17	0.19	0.27	0.23	0.42	0.30	0.42	0.30
10 Weight incr. per mg faeces, mg (*3*:*7*)	1.00	1.01	0.88	0.73	0.70	0.50	0.67	0.48
11 Faeces per cm food, mg (*7*:*5*)	0.17	0.19	0.31	0.31	0.61	0.59	0.63	0.63

Means ± standard errors are given.

Italics refer to means of direct measurements.

Number of individuals: solitary series, males, 22; females, 29.
crowded series, males, 36; females, 21.

Measurement of faeces production and food intake. When fresh shoots were provided, the faeces were collected, dried (at 50° C until constant weight) and weighed. Weighing was collective for all larvae of a series in L1 and L2, and separate (solitary series) or per group of 3 larvae (crowded series) in the other stages. Unconsumed tops of needles were removed from the faeces before weighing. After each change of food, total length of the feeding scars on the needles was measured; this was found to be the most practicable measure of food intake (see Fig. 10). Up to some moment in L3 or L4, the feeding larvae make scars of about the width of their head and of variable length. Lateron, the longitudinal feeding scars take up the total thickness of the needle. Nearly full grown larvae may consume almost the whole needle, leaving only a stump at its base and dropping its top. In the latter case, the length of needle consumed was estimated from the number of almost completely consumed needles, their average length (determined from a sample of intact needles, whose length is fairly constant within shoots) and the length of the remaining stumps and tops.

Especially in the later stages, length of feeding scars is not a precise measure. Since the lumps chewed from the needles leave the gut virtually unchanged, the weight of the faeces may well provide the more accurate measure of food intake (see Kasting & McGinnis, 1962; Waldbauer, 1964).

with a 5-instar growth pattern.

	4th instar ♂		4th instar ♀		5th instar ♂		5th instar ♀	
	soli-tary	crowd-ed	soli-tary	crowd-ed	soli-tary	crowd-ed	soli-tary	crowd-ed
1 Duration, days	*13.2* ±0.3	16.5	*14.2* ±0.4	17.8	*26.1* ±0.4	23.4	*29.3* ±0.5	26.3
2 Weight at end of stage, mg	*41.34* ±0.61	33.25	*49.86* ±0.93	39.90	*119.5* ±2.4	*107.0*	*167.9* ±2.7	*140.1*
3 Weight increase per stage, mg	27.16	21.83	34.57	27.56	78.2	73.8	118.0	100.2
4 Weight increase per day, mg (*3*:*1*)	2.06	1.32	2.44	1.55	3.00	3.15	4.02	3.88
5 Food intake per stage, cm	*34.52* ±1.37	36.80	*37.90* ±1.41	40.10	*183.0* ±7.0	168.0	*261.0* ±6.0	240.0
6 Food intake per day, cm (*5*:*1*)	2.61	2.23	2.67	2.25	7.0	7.2	8.90	9.12
7 Dry faeces per stage, mg	*39.8* ±1.1	37.3	*53.8* ±1.42	50.8	*343.8* ±7.7	316.2	*505.9* ±3.4	465.0
8 Dry faeces per day, mg (*7*:*1*)	3.02	2.26	3.74	2.80	13.20	13.45	17.29	17.97
9 Weight incr. per cm food, mg (*3*:*5*)	0.79	0.59	0.93	0.69	0.42	0.41	0.46	0.46
10 Weight incr. per mg faeces, mg (*3*:*7*)	0.68	0.57	0.64	0.54	0.23	0.22	0.23	0.22
11 Faeces per cm food, mg (*7*:*5*)	1.15	1.01	1.42	1.28	1.88	1.88	1.94	1.94

To obtain data of faeces production and food intake per stage, moulting larvae of the crowded series were transferred from their group to a separate jar, the other larvae of the jar being added again as soon as they too started to moult.

In the crowded series, the quantities measured could not be obtained individually. Since, in the solitary series, the means of the sexes differed considerably in L3 and the later instars, and because the sex ratio happened to be different in the two series, an estimate for males and females in the crowded series had to be made. This was achieved as in the following example:

sum of weights on first day of L4 of 57 larvae in the crowded series (36 ♂, 21 ♀) 671.0 mg
ratio of mean weight of ♂♂ to mean weight of ♀♀ on first day of L4, solit. series 1.08
estimate of average weight of ♂♂ (x) on first day of L4, crowded series
(from: $36\,x + 21 \times 1.08\,x = 671.0$) 11.4 mg
estimate of average weight of ♀♀ on first day of L4, crowded series 12.3 mg

This procedure permits a valid comparison between solitary and crowded individuals, but a possible interaction between sex and the effect of density is blurred. Neither such interaction nor the error introduced by this procedure seemed to be very great, since a comparison between the estimated values and the mean of groups consisting of only one sex (4 groups of 3 males and 2 groups of 3 females) showed no real differences. Moreover, the procedure was tested by estimating the mean weights of male and female pupae of the crowded series, which were known exactly. The estimates differed from the actual means by only 3%.

A consequence of this procedure is that the differences between the series cannot be statistically tested. As a rough measure, the table gives the standard errors of the solitary data.

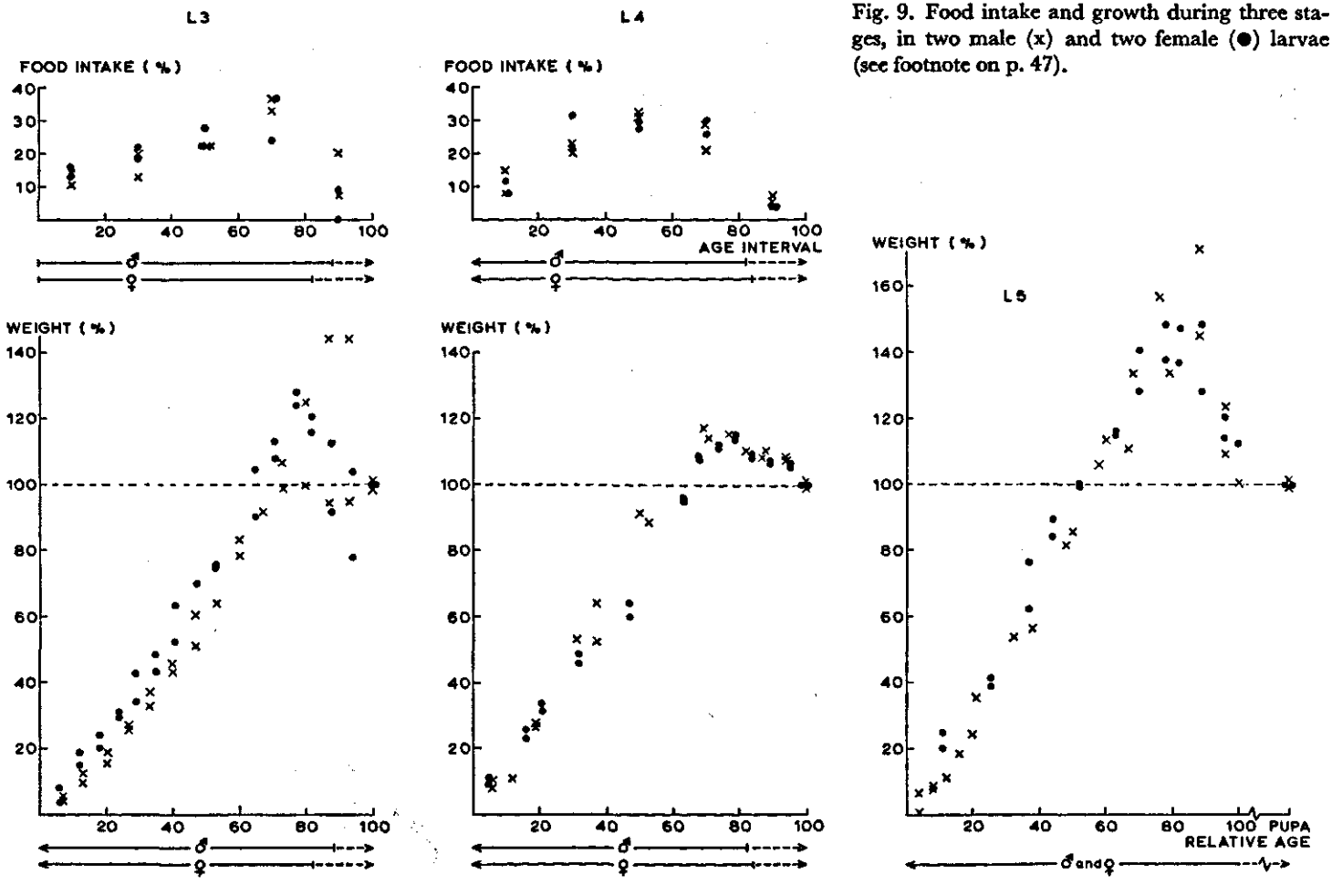


Fig. 9. Food intake and growth during three stages, in two male (x) and two female (●) larvae (see footnote on p. 47).

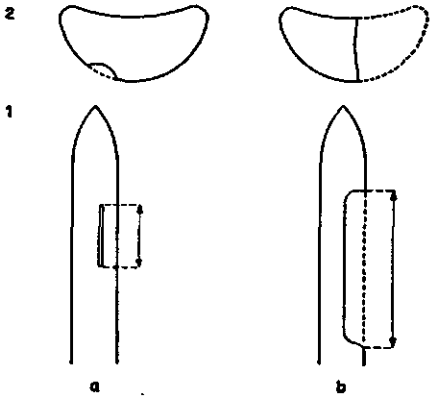


Fig. 10. Feeding scars left by *Bupalus* larvae on pine needles (schematically):
a. first to third instar, b. later instars.
1. length of scars measured as indicated by the arrows.
2. cross-section.

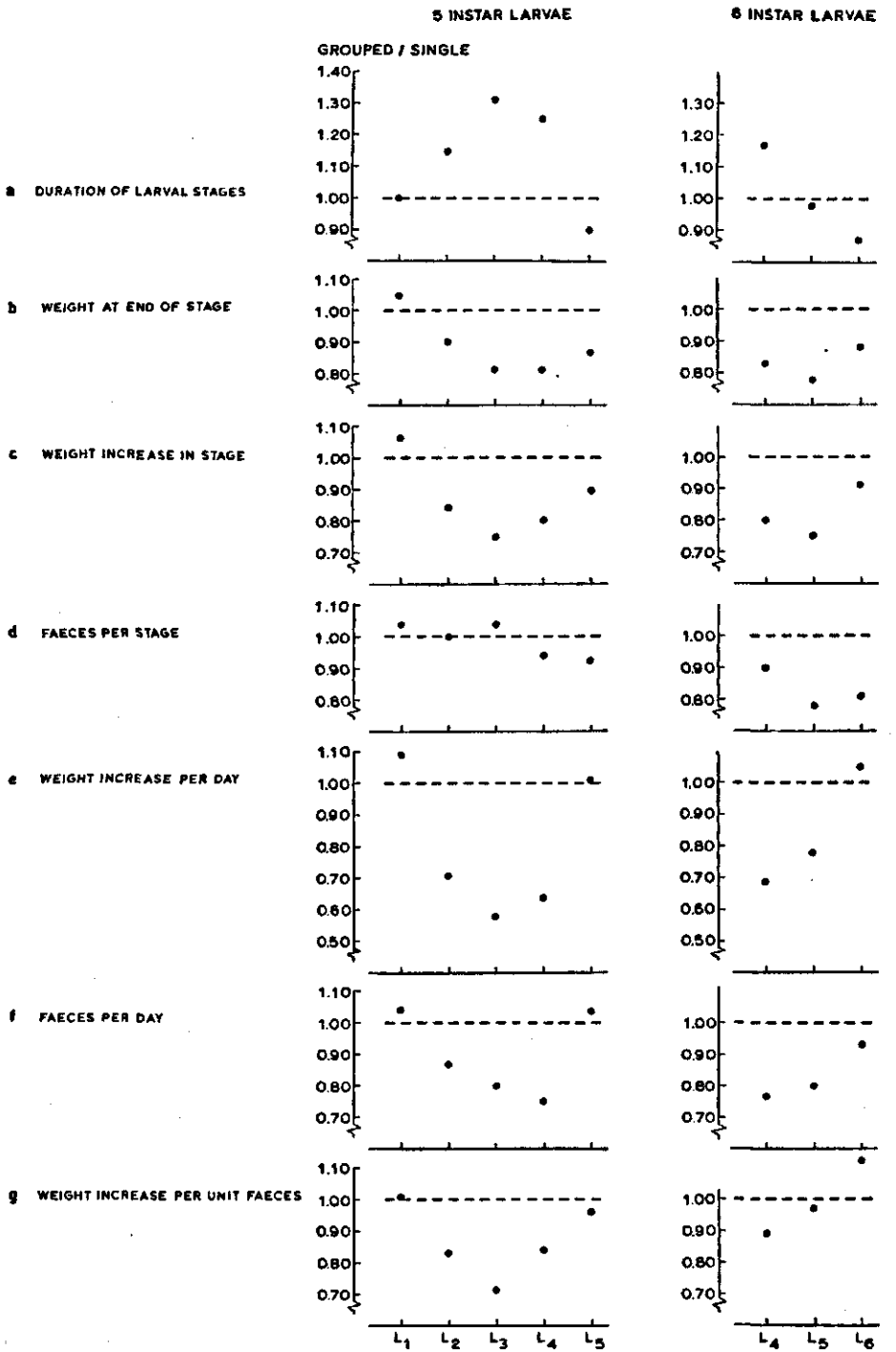
Table 21. Duration (days) of the last (5th) larval stage in relation to date of moulting and larval crowding, in two experiments performed in different years.

Date of moult L4 to L5	Experiment 1[1]		Experiment 2	
	1 larva / jar	3 larvae / jar	1 larva / jar	3 larvae / jar
1 – 5 September			33.0 (n = 7)	22 (n = 1)
6 – 10 September	26.0 (n = 6)		29.8 (n = 12)	24.7 (n = 3)
11 – 15 September	27.8 (n = 28)	24.3 (n = 6)	32.0 (n = 9)	27.9 (n = 8)
16 – 20 September	29.8 (n = 12)	24.8 (n = 12)	36.5 (n = 4)	25.8 (n = 19)
21 – 25 September	27.0 (n = 4)	23.5 (n = 15)	34 (n = 1)	30.6 (n = 11)
26 – 30 September		24.4 (n = 19)		27.0 (n = 31)
1 October and later		28.3 (n = 3)		23.5 (n = 11)

1. Same as in Table 20. Pooled data for males and females.
n = number of observations.

ties are divided by the average duration of the stage concerned (Table 20, lines *4*, *6*, and *8*). In fact, these ratios do not depict the real daily averages, because growth does not proceed linearly during a stage but follows a slightly S-shaped curve with a more or less steep drop at the end of each stage (Fig. 9). For the sake of comparison, however, the ratios are useful. They show that the greatest divergence between the two groups occurred in L3; in L2 and L4 the differences were somewhat smaller, and in L1 and L5 single and crowded larvae had a roughly equal score (Fig. 11). Lines *9* and *10* (Table 20) show that in L2 to L4 the average increase of weight per unit food consumed was lower in aggregated than in single larvae. If the units of food intake are assumed to be the same in both groups, it may be concluded from line *11* that this difference is not due to a reduction in absorption of the ingested food in the aggregated group. Two possible explanations of the observed difference re-

Fig. 11. Growth, food intake, and faeces production in solitary and crowded larvae (data from tables 20 (5-instar larvae) and 23 (6-instar larvae).



main. Firstly, aggregated larvae presumably require more energy to maintain body function during L2 to L5, owing to their longer duration. Secondly, the efficiency of conversion of the absorbed food might be lower in aggregated larvae.

To evaluate the first of these possibilities, the individual data for L3 and L4, of the single series, were used. They indeed showed a negative regression of growth per unit faeces on duration of the stage in three out of four cases (*viz.* in males, L3, and in females L3 and L4).

Now, if duration of the stages is assumed to be the only factor differing between the single and aggregated groups, growth per unit faeces can be calculated for the latter from this regression. The values of Table 22 were obtained. Comparison of the calculated with the observed values shows that the greater part of the difference between the observed values of single and crowded larvae concerned must be ascribed to reduced efficiency of conversion of absorbed food in the latter.

Table 22. Growth (mg) per unit faeces for the crowded larvae of the experiment of Table 20, calculated from the regression of growth per unit faeces on duration of the stage in the solitary larvae.

	Crowded larvae		Solitary larvae
	calculated	observed	observed
Males, third instar	0.61	0.50	0.70
females, third instar	0.64	0.48	0.67
males, fourth instar	0.71	0.57	0.68
females, fourth instar	0.61	0.54	0.64

The same relations between single and grouped individuals developing through six instars were studied in a second experiment conducted in winter under artificial 'summer' conditions (daylength decreasing stepwise from 17 hr in L1 to 11 hr in L6; fluctuating temperature: average diurnal maximum about 21° C, average nocturnal minimum about 13° C). The experimental procedure was as described in the technical explanation to Table 20, with some minor differences.

The results (Table 23 and Fig. 11) show that the differences form the same pattern as that in the experiment of Table 20. Food intake and faeces production are in fair agreement with each other in L4 but not in L5 and L6. Since measurement of the feeding scars tends to be inaccurate for the later stages, it seems best to regard these data with suspicion and to take weight of faeces as the significant measure of food intake. The effect of density in L4 corresponds to that found in L2–L4 in Table 20 (see figs. 11e, f, g); in L6, it is absent, and L5 is intermediate in this respect.

Table 23. Growth, food intake, and faeces production in solitary and crowded female larvae with a 6-instar growth pattern.

	4th instar		5th instar		6th instar	
	solitary	crowded	solitary	crowded	solitary	crowded
1 Duration, days	*10.0* ±0.3	*11.7* ±0.6	*13.4* ±0.5	*13.0* ±0.9	*27.0* ±0.7	*23.6* ±1.6
2 Weight at end of stage, mg	*22.2* ±1.3	*18.4* ±3.5	*59.3* ±3.5	*46.4* ±6.5	*192.5* ±9.9	*167.9* ±20.0
3 Weight increase per stage, mg	*12.7* ±1.0	*10.2* ±2.2	*37.1* ±2.5	*28.0* ±6.6	*133.2* ±6.9	*121.5* ±14.3
4 Weight increase per day, mg	1.27	0.87	2.76	2.16	4.93	5.15
5 Food intake per stage, cm	*21.3* ±1.5	20.5	*46.0* ±3.2	50.5	*183.2* ±6.7	180.7
6 Food intake per day, cm	2.13	1.75	3.44	3.88	6.79	7.65
7 Dry faeces per stage, mg	*15.4* ±0.5	13.9	*56.0* ±2.4	43.5	*414.5* ±20.0	336.6
8 Dry faeces per day, mg	1.54	1.19	4.17	3.34	15.32	14.21
9 Weight incr. per cm food, mg	0.60	0.50	0.81	0.55	0.73	0.67
10 Weight incr. per mg faeces, mg	0.82	0.73	0.66	0.64	0.32	0.36

Means ± standard errors are given. Italics refer to direct measurements.
Average weight at beginning of 4th stage: 9.5 mg in solitary, 8.2 mg in crowded individuals.
Number of individuals in the solitary series 11, in the crowded series 10.
In the crowded series, the larvae were reared in pairs from the moment of hatching, but no measurements were taken before L4 in either series. Individuals in the crowded series were marked with a speck of paint on the head, so that weights were know individually. Development included five or six instars, six-instar females being the only group numerous enough in both series for comparison. In the crowded series only data concerning jars with two six-instar females were considered.

Table 24. Comparison of the effect of density on head width with the effect of density on weight.

Instar	Head width (1 unit = 0.043 mm)			Weight 1st day of instar (mg)			$\sqrt[3]{\text{ratio of weights}}$
	solitary	crowded	ratio	solitary	crowded	ratio	
3	22.1	20.7	1.069	not determined			
4	29.1	27.8	1.048	9.5	8.2	1.159	1.051
5	39.2	36.5	1.072	22.2	18.4	1.206	1.065
6	not measured						

Data from the experiment in Table 23. Means of 11 single and 10 crowded female larvae.

Since head widths were also measured in the present experiment, the ratio of head widths of solitary and crowded larvae can be compared with the ratio of their weights. The results are shown in Table 24. The last column shows that the reduction in head width owing to crowding has about the value that would be expected from the reduction in larval weight, under the hypothesis of isometric growth.

Growth pattern As already mentioned in Part I, density affected the pattern of growth. The evidence has been presented in Table 25 which shows a rather slight, but consistent, reduction in the number of instars under the influence of aggregation. This reduction may have been indirect, since crowding delays development; consequently, under normal photoperiodic conditions, the critical stage for the determination of the number of instars was passed under a slightly shorter photoperiod in the crowded series than in the solitary controls. Short days reduce the number of moults (Part I). The absence of an effect of density on number of instars under constant darkness (although an effect on size did occur) is in accordance with this supposition (Table 26); and the following consideration also favours it.

In crowded larvae, the retardation of development lengthened the period from L1 to L3 (in which the number of moults is fixed) with 5.3 to 12.3 days. As Figure 1

Table 25. Percentages of five and six-instar larvae at different densities in the insectary during summer.

Experiment	1 larva per jar		2 larvae per jar		5 larvae per jar		3 larvae per jar[1]	
	5 instars	6 instars	5 instars	6 instars	5 instars	6 instars	5 instars	6 instars
1	56	44	69	31	74	26		
2	73	27					86	14
3	64	36	87	13				
4	97	3			97	3		
5	64	36			84	16		
6	56	44			73	27		
7	87	13			98	2		
8	69	31			86	14		
9	84	16			93	7		

1. See note 1 under Table 19.
Rearing conditions in all experiments: daylength 16–17 hr in L1, gradually decreasing to 10–12 hr in prepupae; temperature fluctuating, average 15°–17° C.
Number of larvae per series: 43–165.
Incidence of 6-instar larvae significantly greater in solitary than in crowded rearings (sign test).
Since in most crowded series it was impossible to classify each individual according to sex as well as to number of instars, and as in most of the experiments no significant differences were found in sex ratio between single and aggregated series, the data for males and females were pooled.

Table 26. Effect of density on number of instars in darkness under constant conditions.

	1 larva per jar; % with		2 larvae per jar % with	
	4 instars	5 instars	4 instars	5 instars
Temperature 16° C, relative humidity 77%	89	11	86	14
Temperature 24° C, relative humidity 77%	2	98	0	100

About 50 larvae per series.

shows, a difference in date of hatching of about three weeks is associated with a roughly 10% to 35% reduction in the percentage of six-instar larvae in the late-hatching group. Crowding reduces the percentage of six-instar larvae by 14% on the average (Table 25), which can plausibly be ascribed to the delay of development.

Behaviour The behaviour of larvae reared singly and in aggregation was compared for periods varying from one night to several days.

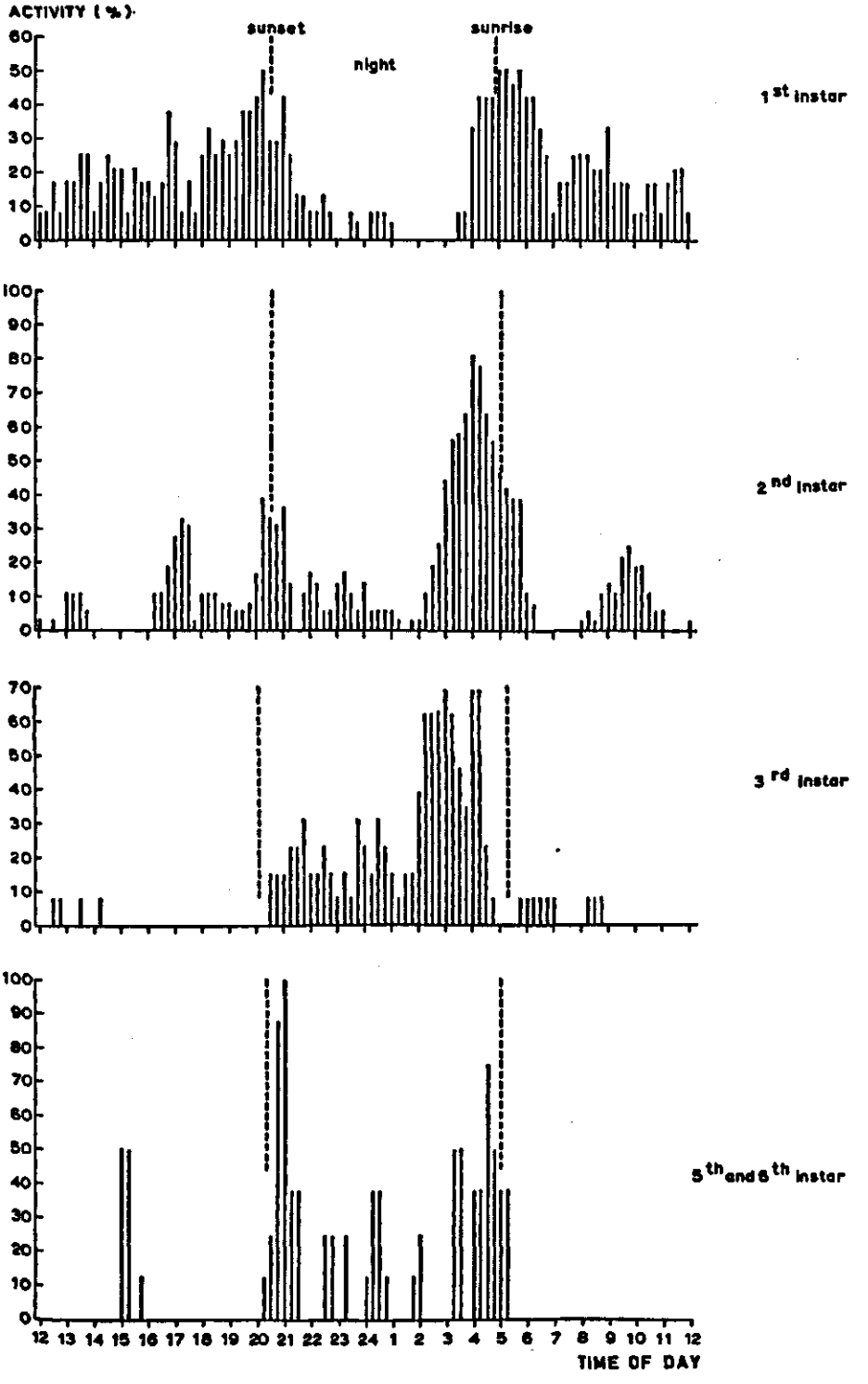
The larvae to be observed were placed on 25 cm long perpendicular pine shoots in the insectary, one larva per shoot in the single, and two or three per shoot in the grouped category. In the latter case, the individuals were marked with specks of paint on the head capsule. Behaviour was recorded continuously by dictating into a tape recorder. Continuous records were supplemented by periodic observations in which the behaviour of each larva was noted once every fifteen minutes to obtain an average of the activity rhythm of a greater number of individuals. Arrangements for observations were made from a few hours to one day in advance, so that the larvae had settled down before the beginning of the observations. At night, dim red light was used (interference filters, wave length of maximal energy transmission 6490 Å, the energy transmitted being halved at each decrease or increase of 81 Å). Red light was used because it is known that several, though not all, insects are insensitive to it (Wigglesworth, 1965). In red light, the behaviour of the larvae seemed to be undisturbed, with a characteristic pattern of alternating periods of activity and resting. Upon sudden illumination with white light in a number of trials the larvae generally stopped their activity abruptly and took on a resting position; this was taken as satisfactory evidence of relative insensitivity to red light.

The observations gave no indication of a difference in the rhythm of activity between solitary and aggregated larvae. The data of both groups are pooled in Figure 12. On the whole, larval activity was concentrated at the beginning and end

Note to Figure 12:

Columns represent combined feeding, walking, and other movements, per 15 minutes, as percentage of total possible activity. Averages of 6 individuals in L1, 12 in L2, 13 in L3, and 2 in L5 and L6.

Fig. 12. Daily rhythm of activity in *Bupalus* larvae (see footnote on p. 56).



of the night, especially the latter. Only in L1 did a considerable amount of activity also occur during the day. The typical rhythm of larvae in L2 and subsequent instars was as follows. When twilight came on, the larvae awoke, left their resting position, and moved about erratically on the shoot for one to five minutes, after which they started to feed. Feeding lasted from 20 to 60 minutes. Between walking and feeding, a few minutes were sometimes spent in palpating movements in which the head was repeatedly moved from side to side. When feeding had stopped, the larva again wandered over the shoot for a couple of minutes, came to rest, cleaned its mouthparts with its thoracic legs, which took one to five minutes, and then reassumed the characteristic resting position in which it was stretched along a needle. After some hours of rest, the same pattern of walking-feeding-walking-cleaning might be repeated. Deviations from this general pattern of course occurred. For instance, walking could last considerably longer and was not necessarly followed by feeding.

The periods of activity of different larvae on either the same or different shoots, were found to be fairly well synchronized.

Table 27 shows that aggregation had almost no effect on activity of the larvae. Both single and aggregated larvae rested during most of the day. Only in L1, crowded larvae seemed to be somewhat more mobile than single individuals and to spend somewhat less time in feeding, which is in contradiction to the fact that no effect of density on growth was detected in L1.

Table 27. Time spent in resting, feeding, and moving by *Bupalus* larvae reared singly and in aggregation.

Instar	Rearing conditions	Number of larvae	Total period of observation in hours	% of time spent by larvae					
				by day			at night		
				in resting	in feeding	in moving	in resting	in feeding	in moving
1	single	5[1]	162	77.5	15.7	6.8	91.8	6.7	1.5
	aggregated	8[1]	189	76.2	11.7	12.1	89.5	4.9	5.6
2	single	7	261	89.4	7.6	3.0	82.0	13.5	4.5
	aggregated	4	195	88.7	7.9	3.4	81.3	13.8	4.9
3	single	5	140	97.9	1.3	0.8	71.1	21.7	7.2
	aggregated	6	168	97.8	0.0	2.2	74.3	20.4	5.3

1. Number of larvae observed during the day: 3.
Aggregated larvae in L1 and L2 were reared in aggregation from hatching to the moment of observation; in L3 they were aggregated a week before observation.
Day: from sunrise to sunset; night: from sunset to sunrise.
Moving: predominantly walking, a small proportion (about 0.5 %) being cleaning of mouthparts and body movements without displacement.

When two larvae met, they usually seemed startled but sometimes walked over each other without any sign of disturbance. When a resting or feeding larva was touched by a moving one, the former was usually the first or the only one to react by more or less vehement swinging movements of the anterior part of the body, as if to throw off an attacker, while the abdominal legs kept hold of the needle. Or the larva might drop several centimeters while spinning a silk thread by which it remained attached to the needle. Some minutes later, it ascended by the thread, which was wound between the thoracic legs, and climbed up on the needle. After either reaction, the larva might resume its resting position or start to walk. Walking not preceded by these reactions also occurred as a result of disturbance. When, in an encounter, both larvae reacted with swinging movements, something resembling wrestling might continue for 20 to 30 seconds, the larvae crawling over each other and swaying, each one trying to throw the other off.

When we return to these encounters in Chapter 3, we shall see that the reactions were aspecific. They are mentioned here because they might raise the activity level of crowded larvae as compared to solitary ones. Although this was not the case in L2 and L3, it may explain the somewhat greater activity of the crowded larvae in L1.

Furthermore, the reactions to encounters could conceivably enhance the dispersion of crowded larvae. The scarce observations on this point do not support this assumption (see p. 85).

2.4 Effect of aggregation on pupae

Size The effect of density on pupal size has already been treated (sections 2.1 and 2.2).

Sex ratio Of 1,940 singly reared individuals, 54.1% were males and 45.9% females, which differs significantly from a 1 : 1 sex ratio. Of 2,110 pupae from aggregated rearings, 48.9% were males and 51.1% females, which differs significantly from the sex ratio in single larvae. The sex ratio of the moths was determined only in some of the experiments; a similar, though insignificant, difference between the two categories was found.

Mortality In pupal mortality, differences between solitary and crowded individuals were not consistent.

Date of emergence of moths Crowding of the larvae caused a delay of adult emergence which was greater in females than in males. Males emerged some days earlier than females, and this difference between the sexes was more pronounced in the aggregated series (Table 28).

Although crowded larvae pupated some 7 to 10 days later than their single controls, retardation of the moment of pupation was not necessarily the cause of the delay of moth emergence. This is borne out by the facts that single rearings showed no correlation between the starting date of the prepupal stage and the date of moth emergence; and that, although six-instar larvae pupated some 7 days later than five-instar ones, there was no difference in the dates of moth emergence between these two groups.

Table 28. Effect of larval density on the date of moth emergence in two experiments performed in different years.

Experiment	Larvae per jar	Date when 50% of moths had emerged	
		males	females
1	1	11 June	13 June
	5	14 June	18 June
2	1	17 June	19 June
	5	19 June	24 June

Rearing of larvae, and storage of pupae during winter, in the insectary.
Differences between larval densities, and between the sexes, are significant (median test).

Chemical composition A preliminary analysis of female pupae from single and aggregated rearings revealed no clear differences in content of dry matter (average 29.4% of fresh weight), carbohydrate (average 4.4% of dry weight) and fat (average 24.3% of dry weight). The nitrogen content showed a small difference of doubtful significance, solitary pupae averaging 10.3% and crowded ones 10.9%[1]. Other analyses were not made.

2.5 Effect of larval aggregation on adults

Size Differences in weight and in some morphological measurements of adults, owing to larval aggregation, are shown in Table 29. It is clear from the ratios in this table that aggregation reduced all linear dimensions to about the same extent. When comparing the ratios of weights with those of lengths, I have tacitly assumed that aggregation does not influence specific weight.

Wing area and wing loading were considered because of the possible relationship between the effects of larval aggregation and adult dispersal, a point which will be treated in more detail in Chapter 4.

The analysis of the difference in wing loading between single and aggregated individuals was pushed slightly further to ascertain whether the wings of aggregated moths were disproportionally large. If the variation in size of the wings were in proportion to variation in overall size of the body, wing area (A) would be related to weight of newly formed pupae (W) as:

$$A = c.\ W^{2/3} \qquad (c \text{ is a constant})$$

or $$\log A = 2/3 \log W + c'$$

1. The analyses were performed by the Centraal Instituut voor Voedingsonderzoek (Central Institute for Food and Nutrition Research) TNO, Zeist.

Table 29. Means of some morphological measurements of the adults from single and aggregated series.

		1st experiment				2nd experiment			
		single	aggregated	significance of difference	ratio single: aggregated	single	aggregated	significance of difference	ratio single: aggregated
Pupal weight, mg	♂	118.3	110.8	$P < 0.001$	$1.07\ \sqrt[3]{\text{ratio}} = 1.02$	125.9	111.0	$P < 0.001$	$1.13\ \sqrt[3]{\text{ratio}} = 1.04$
	♀	164.1	141.8	$P < 0.001$	$1.16\ \sqrt[3]{\text{ratio}} = 1.05$	185.2	146.0	$P < 0.001$	$1.27\ \sqrt[3]{\text{ratio}} = 1.08$
Adult weight, mg	♂	49.8	46.7	$P \simeq 0.15$	$1.07\ \sqrt[3]{\text{ratio}} = 1.02$	(50)[1]	(45)	–	$(1.11)\ \sqrt[3]{\text{ratio}} = (1.04)$
	♀	107.5	90.4	$P < 0.001$	$1.19\ \sqrt[3]{\text{ratio}} = 1.06$	(126)	(97)	–	$(1.30)\ \sqrt[3]{\text{ratio}} = (1.09)$
Wing area, mm^2	♂	450.2	427.4	$P < 0.001$	$1.05\ \sqrt[3]{\text{ratio}} = 1.03$	463.3	413.3	$P < 0.001$	$1.12\ \sqrt[3]{\text{ratio}} = 1.06$
	♀	474.3	421.7	$P < 0.001$	$1.13\ \sqrt[3]{\text{ratio}} = 1.06$	486.4	421.0	$P < 0.001$	$1.15\ \sqrt[3]{\text{ratio}} = 1.07$
Head width, mm	♂	1.94	1.90	$P \simeq 0.01$	1.02				
	♀	1.86	1.78	$P < 0.001$	1.05				
Length of femur III, mm	♂	2.75	2.68	$P \simeq 0.01$	1.03				
	♀	2.99	2.85	$P < 0.001$	1.05				
Wing loading, mg/cm^2	♂	11.0	11.1	not sign.	0.99	(10.8)	(10.9)	–	1.01
	♀	22.6	21.3	$P \simeq 0.03$	1.06	(25.9)	(23.0)	–	1.13

1. Adult weight determined indirectly from pupal weight and the regression of adult on pupal weight, based on a sample of individuals of both series.

Number of individuals: 1st experiment, about 45 in each of the four categories, but adult weight of males and, consequently, wing loading were determined in about 60 per cent of the specimens. 2nd experiment, about 50 in each of the four categories.

Significance of differences: t-test.

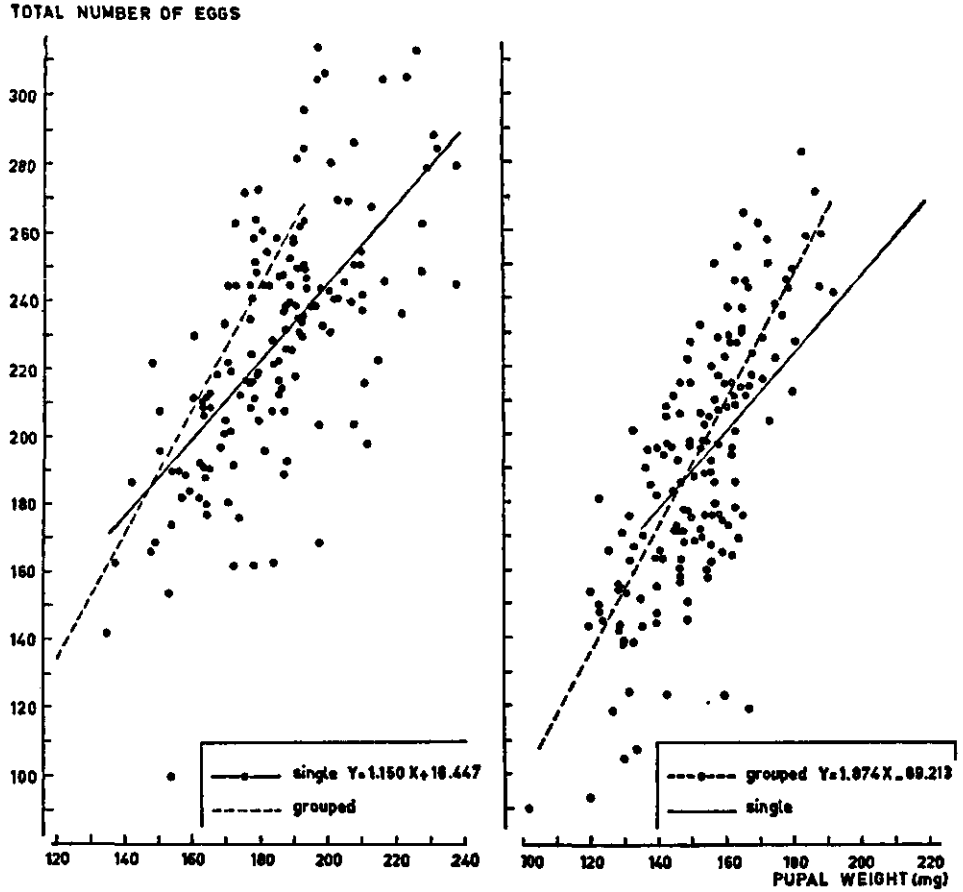
Fresh weight of moths was determined on the day of emergence. Wing area includes the four wings. Head width was measured over and including the compound eyes. Length of femora applies to hindlegs. Wing loading was calculated as the quotient of fresh weight and wing area, for each individual separately in the first experiment and from the means in the second.

Pupal rather than adult weight was used in this relationship to eleminate possible differences due to metabolic losses of weight in and at the end of the pupal stage, which would be irrelevant to the present question.

Regression analysis of data from Table 29 yielded regression coefficients for log wing area on log pupal weight differing only insignificantly from 0.67 in both sexes from single and aggregated cultures. The intercepts on the ordinate did not differ consistently between density categories. Hence, there was no indication of disproportionally large wings in specimens reared in aggregation. It is reasonable to conclude that the higher wing loading of the solitary females is the result of the fact that weight increases with the cube of linear dimensions and wing area with their square.

Fig. 13. Relation between number of eggs and pupal weight, for females from single and grouped rearings.

The data of four experiments were pooled because an analysis of covariance showed no significant differences as to slope and elevation between the four regression lines within each density category. The slope of each of the two regression lines differs significantly from zero, and the two regression coefficients are significantly different from each other ($P < 0.001$).



Lifespan; reproductive capacity Some physiological properties of the adults were studied in experiments in which pairs of moths were reared separately in cages. Adults from single and aggregated rearings showed no differences in longevity, the lifespan being about 12 days for both males and females, or in the proportion of unfertilized females.

The reproductive capacity of females, however, differed clearly. It was determined as the number of eggs laid plus the number of ripe eggs (with a hard chorion) present in the ovaries and oviducts at death (Fig. 13). The averages for females from single rearings were: pupal weight 184.3 mg; total number of eggs, 228.3. For females of aggregated rearings, these values were 151.7 mg and 189.3, respectively, giving a difference in the total number of eggs amounting to 39.0.

Table 30. Comparison of number of eggs produced by females from single and aggregated rearings, for pupal weights from 160 to 190 mg.

Pupal weight in mg	Mean total number of eggs	
	single females	aggregated females
160 – 170	203.2 (n = 19)	209.2 (n = 34)
170 – 180	222.5 (n = 30)	236.4 (n = 11)
180 – 190	229.4 (n = 29)	248.9 (n = 7)

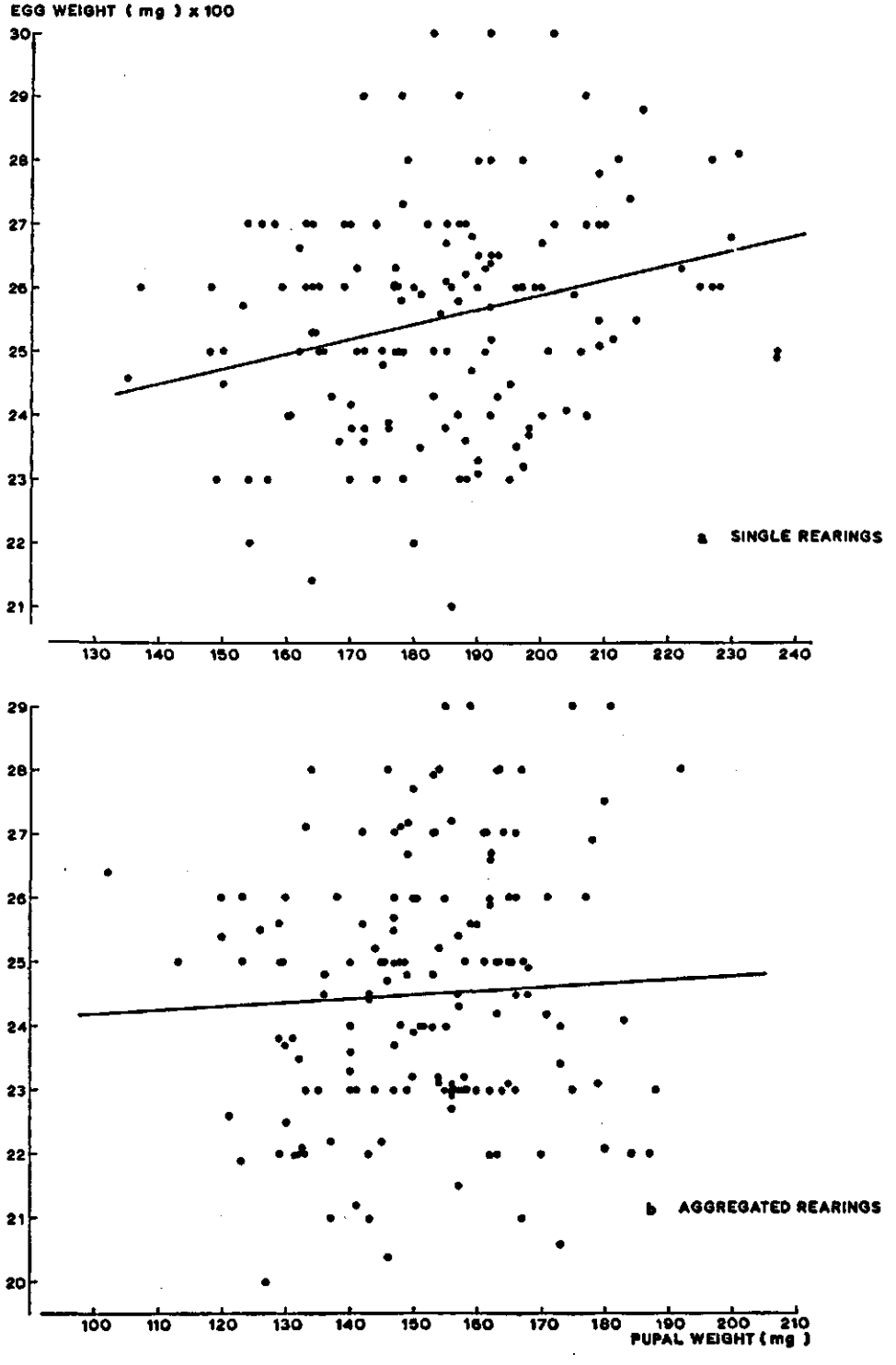
n = number of females.
Significance of differences (three weight classes combined): $P \simeq 0.03$.
Compare Fig. 13.

Table 31. Mean number of eggs laid and mean number of ripe eggs retained at death, in females from single and aggregated rearings.

Experiment	Number of eggs laid in			Number of ripe eggs retained in		
	single rearings	aggregated rearings	significance of difference	single rearings	aggregated rearings	significance of difference
1	187 (n = 61)	180 (n = 43)	0.16	35 (n = 61)	14 (n = 43)	0.03
2	184 (n = 18)	160 (n = 40)	0.10	30 (n = 18)	26 (n = 40)	not sign.
3	207 (n = 37)	169 (n = 46)	0.003	27 (n = 37)	15 (n = 46)	0.11
4	200 (n = 36)	179 (n = 31)	0.05	39 (n = 36)	15 (n = 31)	0.01
total	195 (n = 152)	172 (n = 160)	<0.001	33 (n = 152)	17 (n = 160)	0.003

n = number of females.
Differences tested with Wilcoxon's test; the results of the four experiments combined to obtain the P-value in the last line.

Fig. 14. Regression of egg weight on pupal weight (see footnote on p. 65).



In the crowded group the regression was steeper than in the single one, owing to the relatively low egg production at pupal weights below 150 mg, and to the higher number of eggs above 160 mg. The latter difference is unmistakable (Table 30), but the former is doubtful, since, as the few points available in this range seem to indicate, the regression of the solitary group might well curve downwards at pupal weights below 150 mg. The higher average egg production at pupal weights higher than 160 mg in the crowded group must be considered in relation to egg weight (see below).

In both density categories, most females contained only a few ripe eggs at death but some showed considerable egg retention, the number of these eggs being highly variable. Table 31 shows that the mean number of retained eggs was highest in the solitary group. Consequently, the difference between the two groups with respect to the number of eggs laid was about two-thirds of the difference in total egg production.[1]

In females from single rearings, egg weight was positively correlated with pupal weight (Fig. 14a), but in the crowded group, the regression coefficient did not differ significantly from zero (Fig. 14b). Thus mean egg weight can best be compared without reference to pupal weight (Table 32): crowding reduced egg weight significantly, on the average by 0.01 mg. To explain these differences, I suppose that one

Table 32. Mean egg weight of females from single and aggregated rearings.

Experiment	Mean egg weight (mg) in		Difference (mg)	Significance of difference (t-test)
	single rearings	aggregated rearings		
1	0.251 (n = 34)	0.241 (n = 42)	0.010	P = 0.015
2	0.253 (n = 35)	0.246 (n = 30)	0.007	P = 0.06
3	0.258 (n = 59)	0.255 (n = 43)	0.003	P = 0.43
4	0.262 (n = 17)	0.240 (n = 40)	0.022	P $<$ 0.001
total	0.256 (n = 145)	0.246 (n = 155)	0.010	P $<$ 0.001

n = number of females.
P-value in last line obtained from the combination of those in the four experiments.

1. As shown before (p. 27), single rearings contain a higher percentage six-instar individuals than aggregated rearings, and egg retention is greater in females with six instars than in those with five. These facts explain the difference in egg retention between the two density groups only partially, because a similar difference is still found when six-instar females are left out of consideration.

Note to Figure 14:

a. Single rearings. Data from four experiments, with common regression line $y = 0.0236\,x + 21.145$; $b = 0.0236$ differs significantly from 0.
b. Aggregated rearings. Data from four experiments, as in a, with common regression line $y = 0.0060\,x + 23.643$; $b = 0.0060$ is not significantly different from 0.

Table 33. Summary of rearing experiments on the influence of larval density on viability of the offspring.

		Experiment 1 moths from rearings of 1961				Experiment 2 moths from pupae collected in the field in the spring of 1963	
1962	Larvae reared for tests in next year						
	number per jar	1		5			
1963	Viability tests						
	number of ♀♀	51		32			
	viability of offspring	+		—			
	Larvae reared for tests in next year	┌	┐	┌	┐		
	number per jar	1	5	1	5	1	5
1964	Viability tests						
	number of ♀♀	17	40	11	29	57	45
	actually found viability of offspring	+	+	—	—	+	+
	expected viability from 1963	+	—	+ or + —	— or — —	+	—
	Larvae reared for tests in next year					┌ ┐	┌ ┐
	number per jar	discontinued				1 5	1 5
1965	Viability tests						
	number of ♀♀					34 39	34 30
	viability of offspring					+ +	+ +
						discontinued	

Number of ♀♀: number of females for which the viability of the progeny was tested.
+ and — : significant differences in viability between series of one experiment in one year: + = high viability, — = low viability, — —very low viability.

of the factors determining fecundity is genotypic size. Under crowding, the material available for egg production in individuals of a certain genotypic size is reduced as compared to singly reared specimens, and as a result eggs are smaller.

The smaller regression coefficient of egg weight on pupal weight in aggregated females is plausible, because crowding tends to reduce pupal weight to a greater extent in genotypically large individuals than in small ones (p. 45).

2.6 Viability in relation to larval aggregation of parents

Since Klomp's (1966) data on population trends in *Bupalus* show that high larval densities in some years were followed by high mortality of eggs and young larvae in the next generation, I studied the influence of larval density on viability of the progeny in rearings (Table 33) and in a field experiment.

The rearing experiments were started with single and grouped larvae. During the following spring, the emerging moths within each larval density group were paired. Two pairs were kept per cage in 1963 (Exp. 1) and one pair per cage in the other trials. In the latter case, the offspring of each pair of moths was tested separately, which allowed the assessment of the variation of viability between progenies and the use of more sensitive statistical tests.

In testing viability, I concentrated on eggs and newly hatched larvae, because mortality in these stages is a key factor for population fluctuation in the field (Klomp, 1966). Since differences in viability could conceivably become apparent only under unfavourable conditions, the tests were conducted in the laboratory under conditions differing as to the average percentage survival that they allowed. The eggs were incubated at different temperatures and relative humidities, and the percentage hatched was taken as the index of viability. Young larvae were subjected to a starvation test by keeping them without food for either one or two days after hatching; after these periods, they were placed on pine shoots, and the percentage surviving was assessed ten days later. Some tests were also made with older larvae; in 1963 (Exp. 1) by rearing at the unfavourable temperature of 30° C, and in 1964 (Exp. 2) by rearing at a relative humidity of about 100%.

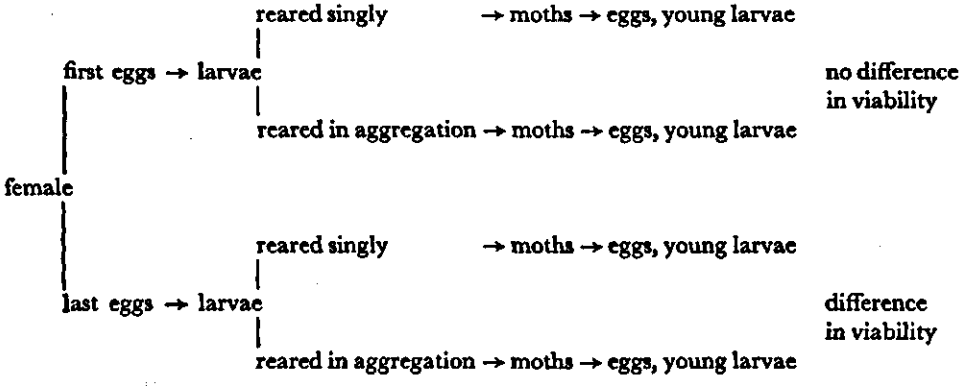
Moreover, larval growth of the progenies was studied in single and crowded rearings. In the following year, the viability of the offspring of these rearings was again tested, to investigate a possible accumulation of the effect of crowding over two generations.

Table 33 shows that the results of these experiments were contradictory. In 1963 (Exp. 1), a significant difference between the two density groups was found. These data have already been published in greater detail (Klomp & Gruys, 1965). In 1964, however, no difference ascribable to crowding of the parents (in their larval stage) could be detected; but the descendants of the crowded rearings in 1962, in Exp. 1 were again of significantly lower viability than the descendants of the single larvae of 1962.

In 1965, again no relation of viability to larval density of the parents was found.

I cannot readily explain these results. A possible explanation of the differences in viability found in Exp. 1 (Table 33) would be that, by chance, individuals of relatively high viability were allotted to the single series of 1962, and individuals of relatively low viability to the crowded series.[1] However, the larvae used for these series were taken from the same stock, and the progeny of a rather large number of females was tested. Therefore, this explanation seems highly improbable.

Another somewhat more plausible but also wholly hypothetical explanation would be that crowding produces an effect on viability of the offspring only in larvae from old females (i.e. females that have laid most of their eggs) and not in larvae from young ones, as follows:



As a matter of fact, eggs from old females were used to make up the rearings of 1962, in contrast to the rearings of the other years. Age of the mother does influence viability: in tests of all offspring of six females, 10% of the eggs failed to hatch, and the 10% that was last to hatch gave rise to distinctly less viable larvae than the 80% that hatched first. But whether the age of the mother also affects the degree of the effect of larval crowding was not tested.

One experiment was performed to test the possible influence of the parents' larval density on viability under field conditions. Two groups of seven 35-year-old Scots pine trees were each provided with about 5000 eggs. The eggs in one group originated from individuals collected in the spring in a forest with high pupal density, and those in the other group were similarly obtained from a forest with low density. In October–November, density of the descending larvae was estimated (with funnel traps) in the two groups as well as in the surrounding forest. From the estimates and the average number of eggs introduced per square metre of ground surface, the mortality percentages of the introduced populations were calculated.

1. Progenies of different pairs of moths may significantly differ in viability, as shown by the experiments of 1964 and 1965, in which the progeny of each pair of moths was tested separately and in a number of different tests.

The unexpectedly high density of naturally occurring larvae interfered to some extent with the evaluation of the results. It was quite clear, however, that mortality in the two groups hardly differed. In contrast to expectation, it was even slightly higher in the individuals originating from the low-density population.

In conclusion, it can only be said that crowding of the parents may possibly, but certainly does not necessarily, reduce viability of the offspring: in three out of four laboratory experiments and in the only field trial, no such effect was found.

2.7 Influence in the field of larval density on growth, relative to the influence of abiotic factors

Growth is modified by larval density, but also (and quite considerably under laboratory conditions) by abiotic environmental factors; in particular by temperature and photoperiod (see Part I).

For an evaluation of the ecological significance of the effect of density, it must be known which factor is of greatest importance in determining growth under natural conditions.

To analyze this point, I used Klomp's data (1966) of larval density and pupal diameter in 14 generations, together with mean temperatures at the Royal Netherlands Meteorological Institute in De Bilt (see p. 9). On the basis of the results discussed in Part I and the present chapter, pupal size was expected to be positively correlated with: *a* mean temperature in April–June, since temperatures above normal make the emergence of the moths and the occurrence of the subsequent instars relatively early, which tends to increase the proportion of six-instar individuals (which are relatively large); *b* mean temperature in July–August, since the proportion of six-instar individuals and pupal size are positively correlated with temperature; and negatively with: *c* larval density.

A multiple regression analysis of mean pupal diameter of females on these three factors showed larval density to be the only significant factor, the influence of both temperatures being negligible. As a matter of fact, a simple regression analysis of pupal diameter on larval density showed that about two thirds of the variance of pupal size could be attributed to regression.

Consequently, it may be concluded that, under natural conditions, variations of pupal size between generations are due mainly to differences in larval density. Temperature and photoperiod, over the rather small range of variation occurring in these factors, are of minor importance.

3 Analysis of the mechanism of the density effect

3.1 Introduction

The results presented in Section 2.1, showing that crowding in laboratory and field experiments produce similar effects, indicate the existence of a causal relationship between population density and growth. The problem of the mechanism underlying the density effect can be split up into three questions: What is the nature of the stimulus that larvae exert upon each other? How is this stimulus perceived? What is the physiological process that leads from the perception of the stimulus to the effect on growth?

This chapter deals mainly with the first of these questions.

Possible stimuli, operating alone or in combination, are:

food contamination (it will be recalled that food quantity was excluded as a possible cause of the effect of density, both in the field and in rearings)

olfactory stimulation

optical stimulation

bodily contact, possibly in combination with the exchange of some secretion.

The first two stimuli are intuitively most likely in a species which seems to be quite inactive, at least during the day, and which in the field shows the density effect over a range of relatively low population densities.

3.2 Food contamination as a possible stimulus

Firstly, the cut shoots used for food in rearings could conceivably dry out more rapidly in crowded than in single series. Hence, in experiments in which fresh shoots were given weekly or fortnightly, the food of crowded larvae might be inferior. However, such an aspecific change of food quality may be rejected as a possible explanation for two reasons: because the density effect persisted with daily changes of food (Table 34), and because the density effect also occurred when singly reared larvae were kept together on fresh shoots on seven inconsecutive nights dispersed over the larval stage (Table 38).

Secondly, a larva could also induce a specific change in the shoot, either by leaving some trace on the needles (secretion; silk threads) or by transmitting, while feeding, some substance to the needles that would subsequently be transported, thus changing the nutritive value of other needles for *Bupalus* larvae. Nuorteva (1958) has described

the effect on the food plant of salivary secretions in Hemiptera. Obviously, the feeding habits of Hemiptera and of Lepidoptera differ fundamentally, and the passage of a secretion to the food plant is less likely for caterpillars than for bugs or aphids. Nevertheless, change in the food plant by *Bupalus* larvae should not be rejected out of hand.

The hypotheses were checked as follows:

a. Two series of larvae, one with one larva and the other with two larvae per jar, were fed with 'contaminated' shoots; two similar series received 'uncontaminated' shoots for food.

The shoots were taken from a group of 10-year-old Scots pine trees, free from *Bupalus* larvae; half of them were left so to provide uncontaminated shoots. Each of the remaining trees was infested with 100 *Bupalus* eggs at the beginning of the experiment and with 30 L2 a month later; these trees furnished the contaminated shoots. The food was renewed once every fourteen days. During the experiment, the presence or absence of larvae was checked on the basis of the frass falling from the trees (for the technique, see Table 16).

The comparison of mean pupal weight, head widths, and speed of development in the four series showed that 'contamination' of food had no effect.

b. A further test of the possible transmission of a '*Bupalus* factor' through the shoots was made by rearing a series of larvae in pairs, one pair per jar and with one shoot, with the two larvae separated by an opaque screen dividing the jar into two equal compartments and the shoot passing through a hole in the screen. A control series was kept in similar jars, also two larvae per jar and one per compartment, but each larva provided with its own shoot. No significant differences in head width and pupal weight were found between the two series.

c. In a field experiment, the following four series of larvae were reared from hatching to pupation on 10-year-old pines: three larvae per tree, one series of singly caged larvae (Series 1) and another of three larvae per cage (Series 2); 42 larvae per tree, one series singly caged (Series 3) and another with three larvae per cage (Series 4). The number of wild *Bupalus* on these trees at L4 averaged three larvae per tree. If the effect of crowding were due to a transportable *Bupalus* factor, the expectation of mean pupal size in the four series would be (1) = (2) > (3) = (4). We found (1) = (3) > (2) = (4), i.e. the only significant differences were due to differences in the number of larvae per cage.

d. To obtain heavier contamination than under Point a, three larvae were kept for one day on each of a number of 5 cm lengths of shoot. The next day, this contaminated food was given to a series of single larvae. For comparison, a second series of solitary larvae was reared on similar twigs which had been kept in jars without larvae for one day. Hence, all larvae received new food each day, whether contaminated or uncontaminated, from the day of hatching until the middle of the last larval stage. Table 34 gives the results. No influence of contamination was found for the males, but some effect was apparent in the pupal weight of the females, particularly in the case of the six-instar growth type. However, contamination can at best

Table 34. Effect on growth of contamination of food.

Number of larvae per jar	Food contamination	Pupal weight (mg) for			Further data on									
		5–instar ♂♂	5–instar ♀♀	6–instar ♀♀	5–instar ♀♀				6–instar ♀♀					
					duration (days) of			head width	duration (days) of				head width	
					L3	L4	L5	L4	L3	L4	L5	L6	L4	L5
1	—	125.8[a] n = 25	187.7[a] n = 10	194.0[a] n = 10	17.8[a]	24.4[a]	35.1[a]	37.5[a]	16.4[a]	14.2[a]	19.0[a]	33.9[a]	30.0[a]	39.8[a]
1	+	122.1[a] n = 20	173.1[a'] n = 17	156.3[b] n = 6	19.4[a]	22.9[a]	33.9[a]	36.9[a]	16.3[a]	13.3[a]	20.0[a]	36.5[a]	30.7[a]	40.2[a]
3		(105.9)[b] n = 48	(133.1)[b] n = 49	(132.5)[b'] n = 21	(27.7)[b]	(27.6)[b]	(26.5)[b]	(32.9)[b]	(25.0)[b]	(19.6)[b]	(20.0)[a]	(28.2)[b]	(28.3)[b]	(37.2)[b]

Head width is in units of 0.043 mm. n = number of individuals.

Within columns, different letters indicate significant differences (t-test; Yates' test); a': significance of difference from a, P = 0.09; b': significance of difference from b, P = 0.10.

Values in brackets, in the third series, could not be ascertained with complete reliability because the three larvae in one jar were indistinguishable. The estimated values given are based on the assumption that the same relationships exist between the various categories of larvae as in the single series. Duration of the stages, in the third series, gives the average for males and females, since the data of the sexes could not be separated. In the solitary series, the differences in the sexes in this respect were only slight.

explain the effect of crowding only partially (contrast the second and the third series of Table 34). Additional data on the growth of female larvae show that contamination of the food did not provoke the characteristic influence of crowding on the duration of the stages. It is clear from the head widths, especially in six-instar females, that contamination did not reduce growth before L5, whereas crowding had already affected growth before L4.

In conclusion, the supposition of some '*Bupalus* factor' passed to and transmitted through the shoot or tree, can be rejected (experiments a–c). Heavy crowding of larvae on a shoot caused a certain reduction in the growth of larvae that subsequently fed on it. But this contamination of the shoot was not sufficient to explain the total effect of crowding on growth (Exp. d). At densities such as occur in the field, no evidence for an effect of contamination of the shoots was found (Exp. a).

Because it does not explain the effect of crowding, the nature of the contamination of the shoot was not investigated. Of the three possiblities mentioned above (physical change, silk threads, and secretion left on the needles), the last seems the most plausible in view of the effectivity of regurgitated substances (see p. 84).

3.3 Olfactory stimulation

This possibility was excluded on the basis of the results of two trials in which the larvae were reared on either side of a double phosphor bronze screen. These screens were fitted between the two parts of jam jars which had been cut in half vertically.

In one experiment, the test series consisted of 30 jars with one larva in each of the two compartments. Screens with a mesh width of 0.19 mm and a thread width of 0.14 mm were used. In the control series, the larvae were kept singly in jars with two similar compartments obtained by fitting an opaque plastic screen between the two halves. Since no significant size differences between the individuals of the two series were found, a further experiment was made with one larva in one compartment, and five larvae in the other (60 replications). During L1 and L2, mesh and thread width were 0.19 mm and 0.14 mm, and during the later stages, 0.45 and 0.20 mm, respectively. In this case the jars of the control series were the same as those of the test series, only one compartment of each jar being used for keeping a single larva. Again, no reduction of growth in the single larvae of the test series was found.

3.4 Optical stimulation

The possibility of optical stimulation was not investigated separately, because several experiments had yielded circumstantial evidence denying it. Firstly, the grouping of larvae only during the day, with separation at night, gave no reduction of growth, whereas aggregation during the night, or part of it, did so (tables 37 and 42). Secondly, existing knowledge about the visual powers of lepidopterous larvae (Dethier, 1942, 1943) does not suggest that *Bupalus* larvae might be capable of making optical distinction between their congeners and larvae of other species, but

Table 35. Effect on growth of aggregation during part of the larval stage.

Series	Rearing conditions	6–instar ♂♂; mean head width of						6–instar ♀♀, mean head width of					
		L1	L2	L3	L4	L5	L6	L1	L2	L3	L4	L5	L6
1	single , L1-prepupa	9.6	15.3[a]	21.6[a]	28.2[a]	36.8[a]	51.7[a]	9.6	15.2[a]	21.4[a]	28.7[a]	38.7[a]	55.6[a]
2	single , L1 and L2	(9.6)	(14.9)	(21.0)	(26.1)[b]	(32.8)[b]	(47.1)[b]	(9.6)	(14.9)	(21.0)	(26.9)[b]	(34.5)[b]	(50.9)[b]
	2 per jar, L3-prepupa												
3	2 per jar, L1	9.6	15.2[a]	21.5[a]	27.9[a]	36.4[a]	51.4[a]	9.6	15.1[a]	21.6[a]	28.8[a]	38.5[a]	55.5[a]
	single , L2-prepupa												
4	2 per jar, L1 and L2	(9.7)	15.1[a]	20.9[b]	27.0[c]	35.0[c]	49.5[c]	(9.7)	15.3[a]	21.2[a]	28.0[c]	36.8[c]	53.8[c]
	single , L3-prepupa												

Means based on 22–32 measurements. Head widths are in units of 0.045 mm.
Within each column, means with different indices differ significantly (Yates' test or t–test, one-sided).
Solid lines: period of aggregation. Dotted lines: head widths in which a reduction in size could be expected.
In brackets: estimates.

nonetheless certain species of larvae can reduce growth in *Bupalus*, and others do not (Table 43).

3.5 Bodily contact

In preparation for the investigation of this point, I determined which larval instars are susceptible to crowding, and whether the density effect is induced in a particular part of the day.

Effect of aggregation in the various instars The effect of continuous aggregation in the larval stage, as we have seen in Chapter 2, becomes manifest in L2, L3, and L4, but not in L1; and in the last stage of continuously aggregated larvae, there is no difference in growth rate with respect to solitary controls.

The first of two additional experiments, in which the susceptibility of various periods of the larval stage were studied, corroborated the lack of susceptibility in L1 (compare series 1 and 3 in Table 35). The results also show that an effect of aggregation could be induced in L2 (contrast series 1 and 4), and was maintained in the later stages when the larva was reared alone. Crowding from the beginning of L3 to the prepupal stage produced a considerably greater effect (contrast series 1, 2, and 4).

Table 36. Susceptibility of the various instars to crowding.

Series	Period of aggregation (2 larvae per jar)	Pupal weight in % of single controls				Significance of difference from series 1
		♂ 4 instars	♂ 5 instars	♀ 4 instars	♀ 5 instars	
1	none	100	100	100	100	
2	whole larval stage	91	69	95	88	+
3	L2	92	92	92	92	+
4	L3	96	89	89	95	+
5	L4	92	91	95	92	+
6	L5	–	88	–	91	+
7	1 day in L3	97	98	99	104	—
8	5 consecutive days in L3	95	83	89	87	+
9	1 day in L2 3 inconsecutive days in L3 1 day in L4	95	83	93	101	+

Number of observations per mean: ♂, 4 instars, 18–50; ♂, 5 instars, 1–5; ♀, 4 instars, 5–26; ♀, 5 instars, 2–12.
+ = significant differences, — = non-significant difference (analysis of variance on original data)
The duration of crowding in series 7–9 can be compared with that in series 2–6 on the basis of the average duration of the stages in isolated individuals: L1, 13 days; L2, 9 days; L3, 14 days (larvae pupating in L4) or 11 days (larvae pupating in L5); L4, 22–26 days (larvae pupating in L4) or 10 days (larvae pupating in L5); L5, 25 days.

In the second experiment (Table 36), all the instars except L1 were tested separately; and the effect of shorter periods of crowding, of either one or five days, was also determined. The experiment was performed under short photoperiods (natural daylength from the middle of August to the end of October, see Fig. 2), which made most of the larvae pupate in L4 and some in L5.

Crowding during any one of the instars from L2 to L5 resulted in a reduction of pupal weight in the same order of magnitude as the reduction due to crowding during the whole larval stage (compare series 3 to 6 with Series 1 and with Series 2). Aggregation during only one day (in L3) had no significant effect, but aggregation for five days, whether consecutive or not, was sufficient (compare series 7 to 9 with Series 2).

Larvae experiencing crowding for the first time during their last instar (Series 6; four-instar larvae in Series 5) showed reduced growth, in contrast to larvae that have also undergone aggregation during the preceding instars (see p. 47-53). Whatever the physiological mechanism behind this phenomenon may be, crowding during the earlier stages apparently induces a lack of susceptibility in the later stages.[1]

Effect of aggregation during different periods of the day In the first attempt to detect possible differences associated with different periods of the day, the larvae of one series were kept in pairs during the daytime and singly at night (the two periods running approximately from 09.30 hr to 17.00 hr and from 17.00 hr to 09.30 hr). A second series received the opposite treatment, i.e. aggregation at night. In both series, these treatments were performed daily in L2 and L3. Sunrise occurred at about 05.00 hr and sunset at about 20.00 hr. In the other stages, the larvae were isolated. Series of single larvae and of continuously aggregated specimens served as controls.

Table 37 shows that diurnal aggregation was without effect, whereas the effect of nocturnal crowding matched that of aggregation during the whole of larval life.

A possible objection to this conclusion is that the difference could result from the inequality of the periods of aggregation in the first and second test series (7½ and 16½ hours per day, respectively), but this problem was eliminated by the results of a more detailed experiment (see Table 42, series 1 to 6). The larvae of series 2 to 6

1. In this context, the following should be added:
a. I obtained some evidence suggesting that the intense mutual stimulation resulting from the crowding of a large number of larvae in one jar for one day, also fails to induce the density effect, like the slighter degree of mutual stimulation provided by the aggregation of two larvae for one day. On the other hand, crowding during a greater number of days (e.g. five, irrespective of whether two or more larvae are grouped) did give an effect. This finding implies that repeated interference with some rhythmical physiological process is necessary to produce a growth reaction. Probably owing to abnormal environmental conditions, the results of the relevant experiment were not sufficiently conclusive.
b. It is clear from Table 38, Series 3, that a growth reduction is induced when aggregation is restricted to the intermoult periods.

Table 37. Effect on pupal weight aggregation during different periods of the day.

Treatment	Mean pupal weight (mg)	
	♂♂	♀♀
single	121.8[a]	182.8[a]
day: 2 larvae per jar; night: 1 larva per jar	123.2[a]	185.1[a]
day: 1 larva per jar; night: 2 larvae per jar	109.8[b]	149.0[b]
aggregated	109.1[b]	140.0[b]

Within each column different letters indicate significant differences (t-test). Number of measurements per mean: 15–32.

were reared singly, except in L3, when each day they were kept in pairs either for 24 hours, only at night, or during different five-hour periods of the night, as indicated in Table 42. At night, work was done under red light. The larvae were weighed during the second moult, i.e. before the treatments were started, and in the third moult, just after termination of treatment. The results were judged on the basis of weight gain in L3 and pupal weight. In the test series, the larvae of a pair could not be distinguished; in mixed pairs, the heavier larva was assumed to be the female. This procedure results in a slight overestimation for females and an underestimation for males.

It is concluded that relatively short periods of crowding during any part of the night are sufficient to induce the density effect to some degree. They needed not even coincide with the main activity periods of the larvae, which occur around sunset and sunrise (see Fig. 12). In fact, crowding around midnight gave the best match with continuously aggregated larvae; and the density effect was possibly less readily induced in the hours around sunrise.

Larval behaviour at night Our next concern is the behaviour of the larvae at night. We have anticipated this point on page 59 in the description of reactions to encounters between larvae. Even on a 25 cm length of shoot, two or three larvae whose activity periods were rather well synchronized showed only a small number of encounters per night and per larva (from none to about five). The contacts seemed quite fortuitous. The larvae might move about for relatively long times in close proximity to each other without ever touching. I observed no evidence of mutual attraction or of repulsion at a distance.

It proved impossible to observe behavioural details of spontaneous encounters, such as the possible release of a secretion. After artificially arranged contacts in Petri dishes, however, droplets of a secretion were found.

It was also found that any kind of disturbance, whether during the day or at night, by a *Bupalus* larva or larvae of other species tested in this respect (the noctuid,

Panolis flammea, and the sawfly, *Diprion pini*), or by a mechanical agent, resulted in essentially the same kind of momentary behavioural reactions.

These results suggested some further lines of research. Firstly, mutual contacts between larvae might directly interfere with feeding. A larva disturbed while feeding might miss the rest of that feeding period, and if it does not compensate for this at some later time, it will ingest less food. Since average food intake per day is lower in crowded than in single larvae (p. 47-53), such a direct effect is conceivable.

Secondly, the excitation caused by contacts might be responsible. Reduced food intake would then be a secondary effect resulting from some primary physiological change.

Thirdly, some secretion or other substance produced by the larvae and transmitted during contacts could be the responsible factor.

Of course, a combination of two or all of these factors might be prerequisite. Let us consider the probability of these possibilities.

Direct interference with feeding This point was tested by comparing the effects of starvation and of grouping on growth (both during a limited number of days).

Table 38. Effects on growth of grouping and starvation.

Series	Treatment	Mean pupal weight (mg)		
		5-instar ♂♂	5-instar ♀♀	6-instar ♀♀
1	control, single	134.1[a]	179.7[a]	204.6[a]
2	starvation	129.0[a]	183.9[a]	204.5[a]
3	grouping	118.1[b]	160.5[b]	185.0[a']

Within each column different letters indicate significant differences; a': P = 0.08.
Number of measurements per mean: 5-instar males and females: 15–26, 6-instar females: 6–8.
Details of treatments. In Series 2, the larvae were reared singly and starved for two, three, and two inconsecutive days, in L3, L4, and L5, respectively. Larvae completing six instars were starved for an additional day in L6. This makes seven or eight days of treatment for a larval stage lasting about 100 days. For sake of uniformity of treatment, the days of starvation were fixed in relation to the preceding moult, e.g. on the fourth and the eight days after the second moult, etc. One day of starvation consisted of a sojourn of about 24 hours in an empty glass tube; each larva had its own tube throughout the experiment.
In Series 3, grouping (in groups of five) was applied for the same number of days and on identically fixed ages as in Series 2; apart from the seven or eight treatment days, rearing was single.
The larvae of Series 1 were reared singly and got no special treatment.

Table 38 shows that the effect of grouping cannot be caused by direct interference with food intake, since, unlike aggregation, even complete starvation (during the days of treatment) did not reduce growth.

Excitation The effect of excitation (and, to some slight extent, of the transmission of a fluid spat out by the larvae) was evaluated by mechanical disturbance, with a brush, of singly reared larvae. The treatments were given during the day in one of the test series and in the evening in the other (Table 39).

Since the same brush was used to stimulate all larvae of a test series, it occasionally became moist because some of the larvae reacted with oral ejection of droplets. Hence 'excitation' and 'transmission of a substance' were not strictly separated in this experiment.

As Table 39 shows, neither of the two test series matched the reduction of growth due to crowding, although some slight (and insignificant) effect of nocturnal disturbance can be seen for the males. No correlation was found between the average intensity of the immediate behavioural reaction to stimulation and pupal weight.

Since these negative results were obtained with excitation as the main factor, we continued our search in the direction of the 'substance'.

Table 39. Effect on growth of artificial disturbance.

Series	Treatment	5–instar males		5–instar females	
		head width L4	pupal weight	head width L4	pupal weight
1	single	34.2^{a}	126.8^{a}	35.5^{a}	183.3^{a}
2	disturbance at day	34.3^{a}	127.3^{a}	35.5^{a}	180.7^{a}
3	disturbance at night	33.7$^{a'b}$	122.8$^{a''}$	35.4^{a}	185.9^{a}
4	aggregated	(33.2)^{b}	114.1^{b}	(35.2)^{a}	160.7^{b}

Head width is in units of 0.043 mm and pupal weight in mg.
Within each column, different letters indicate significant differences (t–test, Yates' test); a': significance of differences from series 1 and 2, $P \simeq 0.14$; a'': significance of differences from series 1 and 2, $P \simeq 0.2$.
In brackets: estimates; males tend to be underestimated and females overestimated (see p. 77).
Number of measurements per mean: 15–30.
The treatment period ran from the beginning of L3 to the middle of L4, over a period of 38 days, on 30 of which the larvae of series 2 and 3 were disturbed once daily between 10.30 hr and 16.30 hr (Series 2) and between 19.30 hr and 23.00 hr (Series 3). Sunrise was between 05.20 and 06.20 hr and sunset between 18.45 hr and 20.10 hr.
The procedure of treatment was: location of the animal on its shoot with as little disturbance as possible, and noting its behaviour; disturbance by a sudden gentle touching of the head and other parts of the body, together lasting about one minute. Notes were made on the immediate behavioural reactions. Dark red light was used in the evening.
The controls consisted of one series (1) of single untreated larvae and one series (4) in which the larvae were kept in pairs during the 30 days of treatment.

Exchange of a substance during contacts Singly reared larvae kept for a certain time on shoots smeared with ground *Bupalus* larvae showed a reduction of growth, even slightly more than that produced by grouping in a control series (Table 40). It is therefore probable that some substance produced by the larvae is essential for the induction of the density effect.

In an attempt to localize the source of this substance, I concentrated on the mandibular and labial glands (Fig. 15). The results were negative. The labial glands, which generally secrete saliva in insects, are modified in caterpillars to secrete silk. The function of the mandibular glands, which occur commonly in lepidopterous larvae (Bordas, 1909; Dauberschmidt, 1934), is uncertain. Bordas (1909) suggested a dual function, digestive and defensive, in certain lepidopterous larvae. Henseval (1897) found no wood-attacking properties of the copious amount of mandibular

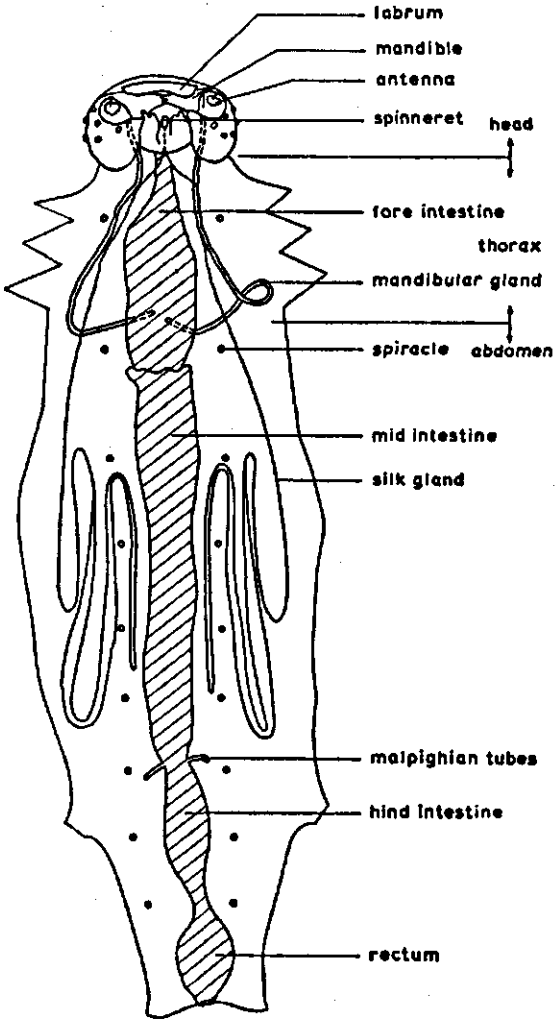


Fig. 15. Silk and mandibular glands in L5 of *Bupalus*, ventral view.

Table 40. Effect on growth of food contamination consisting of pounded larvae.

Series	Treatment	5–instar ♂♂		5–instar ♀♀	
		head width L4	pupal weight (mg)	head width L4	pupal weight (mg)
1	control, single	33.7[a]	114.6[a]	34.7[a]	152.3[a]
2	smeared shoots	33.0[b]	101.9[b]	34.2[a]	139.3[a']
3	control, grouped	(33.4)	107.9[a']	(34.6)	144.5[a'']

Head width is in units of 0.043 mm.
Within each column different letters indicate significant differences (head widths, Yates' test; pupal weight, t–test). a' significance of difference from a, $0.05 < P < 0.10$; a'': significance of difference from a, $P = 0.14$.
In brackets: estimates (see p. 77).
Number of measurements per mean: 11–38.
Details of treatments. The larvae of Series 2 were reared singly. During five inconsecutive four-day periods, each larva was kept on a shoot smeared with a pulp made from five larvae pounded with a few drops of water. During the four days, the smears dried out rather than rotted.
The single controls (Series 1) received similar but untreated shoots during the five periods, as did the larvae of Series 3, which were grouped (2 larvae per jar) during the periods of treatment of Series 2. The experiment was performed in the spring under artificial conditions, which might explain the unusually small effect of grouping.

gland secretion produced by the larvae of *Cossus cossus*, but since he observed it to inhibit growth of an insect-attacking fungus, he suggested a defensive function. Recently, Wronizewska (1966) found evidence supporting its salivary function in the larva of the wax moth, *Galleria mellonella*. Mandibular gland secretions are known to act as coordinators of interindividual relationships in several species of social insect (for instance in the honeybee: Butler, 1964; meloponine bees: Lindauer, 1967; bumble bees: Stein, 1962; ants: Wilson, 1963), which suggests that they might also play a role in the causation of the density effect.

The mandibular glands of *Bupalus* contain a colourless fluid which dissolves readily in a mixture of ethyl alcohol and diethyl ether but not in water or in alcohol alone. The effect of gland contents was studied as follows. Silk and mandibular glands were extirpated and homogenized in a 1 : 1 mixture of ethyl alcohol and diethyl ether. After centrifugation, the fluids were spread evenly on the needles of the shoots on which two series of single larvae were tested (one with respect to the silk, the other to the mandibular gland). The solvent was allowed to evaporate before the larvae were placed on the shoots. Freshly treated shoots were given eight times at weekly intervals, in L3 to L5; in each of these treatments, one pair of glands was used for each larva in the test series. Controls consisting of single and grouped larvae were reared on shoots treated similarly with the solvent alone. No effect on growth (head width of L4; pupal weight) was found in either of the two test series.

On the basis of Loher's (1961) finding that a volatile substance secreted in the epidermis of the adult male desert locust, *Schistocerca gregaria*, accelerates the maturation process of young adult locusts, a second experiment was performed. Loher found a 1 : 1 mixture of diethyl ether and a paraffin oil (Shell Risella 17) to give the most convenient fluid for extraction; the active material could be preserved for months in the oil after evaporation of the ether. I made similar extracts of *Bupalus* and applied them to dummy larvae, having the same size as *Bupalus* and consisting of thick blotting paper, which were glued onto the needles of the shoots, thus simulating the natural presentation of the hypothetical substance (i.e on the larvae, in mutual contacts).

New shoots carrying three freshly treated dummies each, were given twice weekly (17 times in all) from L2 to L5–L6. The following extracts were used to impregnate the dummy larvae:

a. Whole, freshly killed larvae were shaken for 5 hours in a 1 : 2 mixture of paraffin oil (Shell Risella 17) and diethyl ether. The ether was then allowed to evaporate for 18 hours at room temperature. After centrifugation, the supernatant fluid was applied to the dummies. One larva was extracted for each larva in the test series.

b. Heads plus thoraces (containing the mandibular glands) of freshly killed larvae were homogenized in a 1 : 2 mixture of Risella oil and ether. The suspension was

Table 41. Effect on growth of food contamination consisting of pounded parts of larvae.

Series	Treatment	5–instar ♂♂		5–instar ♀♀	
		head width L4	pupal weight (mg)	head width L4	pupal weight (mg)
1	single, untreated	33.0[a]	113.1[a]	35.1[a]	164.5[a]
2	single, smears of head and thorax on shoot	33.0[a]	103.5[b']	35.0[a]	152.0[c']
3	single, smears of abdomen on shoot	32.6[a]	99.1[b]	35.6[a]	150.4[c]
4	in pairs, untreated	(30.7)[b]	96.7[b]	(33.2)[b]	139.4[b]

Head width is in units of 0.043 mm.

Within each column, different letters indicate significant differences (t–test); b': significance of difference from Series 4, $P \simeq 0.15$; c': significance difference from Series 1, $P \simeq 0.07$.

In brackets: estimates (see p. 77).

Number of measurements: ♂♂, 27–29; ♀♀, 31–36.

Details of treatments. Pulps of heads plus thoraces (Series 2) and abdomens (Series 3) of larvae of the same age as in the test series were made by pounding the parts with a few drops of water. One part was pulped per test larva. Immediately after being made the pulps were applied to the shoots of the test larvae. About five drops of the pulp were placed on needles of each shoot and one drop was put against the head of the test larva to maximize the chance of its contacting the pulp. This treatment was repeated 12 times at 3 to 6 day intervals, in L2 to L5. The contaminated shoots were left with the larvae until the next treatment. In the control series (1 and 4) the treatments with larval pulp were simulated with distilled water.

shaken for five hours, after which evaporation and centrifugation were done as under *a*.
c. Abdomen, procedure as under *b*.

The larvae of the fourth test series received shoots with a freshly killed *Bupalus* larva glued to one of the needles. Single and grouped larvae on untreated shoots, and single larvae on shoots with dummies soaked in the extraction fluid, were reared as controls. Growth was gauged from head width and pupal weight.

Neither the extracts not the presence of dead larvae on the shoots had an effect on growth.

Nevertheless, both the heads plus thoraces and the abdomens, when simply pounded with a few drops of water and applied as smears on the shoots occupied by single larvae, gave an unmistakable reduction of growth (Table 41). This corroborates the conclusion drawn from Table 40: *Bupalus* larvae apparently contain a substance that

Table 42. Effects on growth of aggregation during different periods of the night and of two kinds of artifical disturbance.

Series	Treatment	Mean increase of weight in L3 (mg)		mean pupal weight (mg)	
		♂	♀	♂	♀
1	single (control)	9.8[a]	11.0[a]	126.9[a]	170.4[a]
periods of aggregation:					
2	whole day (24 hr)	(7.9)[b]	(9.5)[b]	113.4[b]	152.4[b]
3	whole night (17.30 hr – 08.30 hr)	(7.8)[b]	(9.1)[b]	116.6[b]	152.3[b]
4	first part of night (17.30 hr – 23.30 hr)	(8.7)[c]	(9.5)[b]	113.7[b]	152.2[b]
5	middle of night (23.30 hr – 03.30 hr)	(8.1)[b]	(9.2)[b]	117.3[b]	153.8[b]
6	last part of night (03.30 hr – 08.30 hr)	(8.7)[c]	(9.8)[b]	114.5[b]	163.8[a]
kinds of artificial disturbance:					
7	mechanical	9.8[a]	10.9[a]	128.3[a]	175.2[a]
8	mechanical + secretion	8.2[b]	8.4[c]	127.2[a]	164.6[a1]

1. Difference between series 7 and 8: P = 0.07 (two-sided).
Within each column, different letters indicate significant differences.
In brackets: estimates.
Number of measurements per mean: 13–25.
During the period of treatment (L3), sunrise and sunset were at 05.40 hr and 19.40 hr, respectively.
Details of treatments. Series 2 to 6: see p. 77. Series 7 en 8. The larvae of Series 8 were reared singly; in L3, they were disturbed twice daily, between 03.00 hr and 08.00 hr, with a fine brush wetted by making larvae of a separate batch regurgitate. After treatment of two or three larvae of Series 8, the procedure of wetting the brush was repeated. The first gentle touches to stimulate a test larva were applied to the head, the subsequent ones to the rest of the body, the whole stimulation lasting 30 to 60 seconds. The work was done under dark red light. The larvae of Series 7 were reared singly and received a similar but solely mechanical stimulation, with the blunt end of pine needles. A fresh needle was taken for each larva to avoid transmission of possible active substance from one larva to another.

can reduce growth in their congeners. The active substance is not restricted to either the head and thorax or the abdomen of the larvae. Moreover, it is not present on the outside of the body, since freshly killed *Bupalus* had no effect. It therefore seemed worth-while to submit the fluid gut contents, which are sometimes regurgitated as a reaction to disturbance, to a final test.

This experiment was conducted in combination with the detailed test concerning the period of the night in which the effect of grouping is induced (see p. 76-77). Hence, at the beginning of the experiment, I did not have a sufficient basis for choosing the most appropriate period for artificial stimulation. Of the two peaks of larval activity, the one at the end of the dark period was chosen (cf. Fig. 12). The treatment with regurgitated gut contents was applied during L3. The larvae were weighed during the second and the third moults, and the effect of the treatments was judged from gain of weight in L3 and pupal weight.

Stimulation with regurgitated fluid clearly reduced gain of weight in L3. Purely mechanical stimulation, to the contrary, showed a complete match with the untreated series (Table 42; series 1, 6, 7, and 8). Reduction of growth in L3 was even greater in Series 8 than in Series 6.

In L4 to pupation, however, all of the initial weight reduction in Series 8 was eliminated in males, and about half of it in the females. This is in contrast to the usual situation (see series 2 to 5, Table 42 and tables 35, 36), but partially agrees with the results of the females of Series 6. The only explanation for this discrepancy seems to be that the choice of the period of treatment must have been unfortunate, i.e. the induction of the effect of density occurs in the middle of the night or earlier, rather than later in it.

At any rate, there is strong evidence that a substance regurgitated by the larvae when disturbed is a necessary stimulus for the induction of the density effect. Purely mechanical stimulation cannot produce the effect. The nature of the active substance is unknown.[1]

1. The following observations on regurgitation were made. The fluid was spat out after artificial stimulation, and droplets of it were found after arranged contacts and the subsequent 'wrestling' of two larvae in a Petri dish. When drops protruded from the mouth, the mandibles were opened wide and the other mouth parts made movements to work the drops out. The fluid was colourless to green; in the latter case, numerous minute particles were suspended in it. Regurgitation of the big chunks of food with which the gut is crammed after feeding was never observed.

The colour of the fluid and, to a lesser extent, the readiness to regurgitate, varied with time of day. After one stimulus with a brush applied in the neck region between 11.00 hr and 14.00 hr, 65% of the larvae regurgitated, and around midnight 90% did so; intermediate scores were obtained around 06.00 hr and 18.00 hr. At about 06.00 hr, about 80% of the regurgitated drops were colourless, and at about 18.00 hr about 90% were green, scores being intermediate at about midnight and midday. These changes undoubtedly reflect the daily rhythm of food digestion.

Bodily contact and larval population density in the field Since I have concluded that the density effect is induced by a substance regurgitated by and transmitted between larvae during contacts, I must now consider the probability of larval contacts in nature.

Just after hatching, encounters between larvae must be frequent, irrespective of the population density, because the eggs in a row hatch within a short time. But aggregation during L1 is ineffective (p. 75) and the larvae disperse in this stage.

To obtain information about this dispersal, we imitated the natural situation by mounting pine shoots in a flat arrangement and provided them with eggs. In these experiments (and in the similar ones mentioned below) the shoot area was great relative to the larvae's radius of action. When left undisturbed for four to eight days after hatching, first instar larvae were found to have travelled an average distance of 9 cm in a straight line from their origin.

Similar trials were made with larvae of L2 to L5, by placing groups of 5 to 10 close together in the centre of the shoot arrangements and noting their position some days later. In all cases, the animals were found to have radiated from the point of release, the average distance to this point becoming greater with increasing age. No tendency to separate as far as possible was noted. Apparently, there is no mechanism of mutual attraction, and dispersal is random.

In the field, mortality of young larvae is high; according to Klomp (1966) it amounts to 54% on the average in L1. This undoubtedly increases the average distance between survivors.

Thus there is satisfactory evidence that dispersion from the initial aggregative distribution is great enough for the over-all population density to act as a determinative factor with respect to the degree of mutual interference.

Another question is, whether the activity and area visited by the larvae are such as to permit the occurrence of effective mutual interference, at least at the higher and intermediate densities in the field.

Concerning this point, I would refer first of all to the fact that a rather low number of days of aggregation was found sufficient to induce the density effect in our experiments, and to our observations suggesting a low frequency of encounters among larvae under experimental conditions (p. 76 and 77, respectively). Evidently, a limited number of contacts spread over several nights is sufficient for the induction.

Secondly, we made some observations on the areas visited by solitary larvae in L4 and L5 placed on flat shoot arrangements under shelter in the garden of the Institute. During a period of four to eight days for each animal, an indication of the area visited each night was obtained from the larva's position in the morning and the location of the faecal pellets it had dropped on a sheet of paper smeared with tanglefoot. During these observations, care was taken not to disturb the animals. From these data the fraction of the available area visited in four to eight days was estimated and expressed in number of yearling shoots present, to permit comparison with Klomp's larval density figures.

The average area visited in four to eight days by single L4 and L5 individuals

contained 52 yearling shoots (six replications; standard error = 9), the sum of the areas visited per night being much greater. Klomp's estimates of average larval density in the corresponding stage range from one larva per 12 to one larva per 500 yearling shoots in years of high and low population density, respectively; and the average over all years studied amounts to one larva per 44 shoots.

It therefore seems reasonable to conclude that effective mutual interference can occur in the field, at least at medium to high population densities.

3.6 Effect of other species on growth of Bupalus.

A possible interference of other species of larvae with growth of *Bupalus* is important in relation to the nature of the stimulus inducing the density effect, but also in relation to the interpretation of its function.

The species of larvae tested in this respect were the Lepidoptera, *Ellopia prosapiaria* (Geometridae), *Panolis flammea* (Noctuidae), and *Dendrolimus pini* (Lasiocampidae), and the sawfly, *Diprion pini* (Diprionidae).

All four species inhabit Scots pine. *Ellopia, Panolis* and *Dendrolimus* larvae have solitary habits. The larvae of *Diprion pini* are gregarious (but they can be reared satisfactorily in isolation, at least from L2 onward). *Ellopia* and *Diprion pini* are known to have a diurnal rhythm of activity, with feeding and other activities occurring mainly at night; in the other species, activity is not restricted to a special part of the day (Herrebout et al., 1963; Prop, 1960).

Concurrently with the experiments described below, we made some observations on half and full grown larvae of *Panolis* and *Diprion*, and on L1 of *Dendrolimus*. They all fed during the day as well as at night, without a clear preference; feeding is the main component of activity. The difference with respect to rhythm of activity in *Diprion pini* between Prop's results and mine might be due to the isolation of the larvae from their colonies in my experiments.

Dendrolimus differs from the other species as well as from *Bupalus* in its occurrence on the stem of the shoot during part of the 24–hour cycle. At night, however, it was almost always found on the needles: there was a definite shift of the larvae from the stem or the basal part of the needles to their distal part at sunset, and a movement in the opposite direction at sunrise.

Hence, the behaviour of the species tested does not a priori preclude their interference with *Bupalus*.

In Table 43 (Experiment a) the growth of individual *Bupalus* larvae reared, from the time of hatching, together with one *Ellopia* of the same age (Series a2) is compared with that of singly grown *Bupalus* (Series a1). *Ellopia* either pupated or entered diapause as a half grown larva during L4 of *Bupalus*. Consequently, rearing conditions for *Bupalus* in Series a2 can be considered to have been single from about L4 to pupation. A control series of grouped *Bupalus* was not used in this experiment.

In Experiment b shown in the table, one *Ellopia* (L3) or one *Dendrolimus* (L1) were added to single L3 of *Bupalus* (series b2 and b3, respectively). All *Ellopia* larvae

Table 43. Effect on growth of *Bupalus* of aggregation with larvae of other species

Series	Larvae per jar	Pupal weight of *Bupalus* (% of single controls)			
		5–instar ♂♂	6–instar ♂♂	5–instar ♀♀	6–instar ♀♀
Ellopia:					
a1	1 *Bupalus*	100[a]		100[a]	
a2	1 *Bupalus* + 1 *Ellopia*	93[b]		89[b]	
Ellopia and *Dendrolimus:*					
b1	1 *Bupalus*	100[a]		100[a]	
b2	1 *Bupalus* + 1 *Ellopia*	91[b]		93[b]	
b3	1 *Bupalus* + 1 *Dendrolimus*	103[a]		102[a]	
b4	5 or 6 *Bupalus*	89[b]		87[c]	
Panolis:					
c1	1 *Bupalus*	100[a]	100[a]	100[a]	100[a]
c2	1 *Bupalus* + 1 *Panolis*	93[b]	98[a]	96[a]	98[a]
Panolis and *Diprion:*					
d1	1 *Bupalus*	100[a]		100[a]	100[a]
d2	1 *Bupalus* + 1 *Panolis*	100[a]		95[a']	92[a''']
d3	2 *Bupalus* (control of d2)	89[b]		82[b]	75[b]
d4	1 *Bupalus* + 1 *Diprion*	101[a]		96[a'']	95[a]
d5	2 *Bupalus* (control of d4)	91[b]		86[b]	81[b]

Within the colums of each of the four experiments, different indices indicate significant differences; a', a'', and a''', significance of differences with a: P = 0.15, 0.20 and 0.11, respectively; t–test on original data.

entered diapause during L5 of *Bupalus* in this case, and *Dendrolimus* did so about the pupation time of *Bupalus*. A series of grouped *Bupalus* (Series b4) gives an impression of the maximum intraspecific influence in this case.

Since *Panolis* larvae occur earlier in the year in nature than *Bupalus* (they hatch in May and pupate in July), the larval period of the *Bupalus* used in Experiment c was artificially advanced (see Part I, Chapter 2). From the middle of L1 of both species, one *Panolis* larva was grown together with each *Bupalus* larva of Series c2; after pupation of *Panolis* during L4 of *Bupalus*, the latter was reared singly. Again, no direct check on the intraspecific effect is available.

The effect of *Panolis* was retested along with an additional series to ascertain the influence of *Diprion pini*. In Series d2 one *Panolis* L2 was added to each *Bupalus* in the middle of the latter's L2. Grouping lasted up to the beginning of L4 of *Bupalus*, when *Panolis* pupated. The *Bupalus* larvae of the control series (d3) were kept in pairs in the same period, and reared singly during the rest of the larval stage. In Series d4, one

Diprion L2 was added to each *Bupalus* in L3 and grouping lasted to the end of the larval stage of *Bupalus*. In the control series (d5), *Bupalus* was kept in pairs in the same period.

In the experiments with *Panolis* and *Diprion*, head widths of L3 to L5 *Bupalus* were measured. They either showed no effect of aggregation shared with *Panolis* and *Diprion*, or a smaller effect than that expressed in the pupal weights.

Summarizing the results, it can be said that the species tested showed divergent interference with growth of *Bupalus*. *Dendrolimus* had no effect, and *Ellopia* reduced growth of *Bupalus* to about the intraspecific level. *Panolis* and *Diprion* were intermediate; they gave only a slight effect, *viz.* about one quarter of the intraspecific reduction.

I conclude from these results that the density effect in *Bupalus* has a limited degree of unspecificity. Since, as has been mentioned before (p. 77-78), interspecific and intraspecific disturbances release the same immediate behavioural reactions, the different effects on growth of the four species tested form additional evidence against mechanical stimulation as a possible cause of the density effect.

The fact that *Bupalus* is by far the most abundant species of phytophagous larva in pine forests in The Netherlands explains why, given a 'limited unspecificity', a correlation of growth of *Bupalus* with its own density is found in nature.[1]

1. Klomp's (1962) data show the fluctuations of population density of some seven species of pine-inhabiting larvae to be asynchronous, and the mean levels of abundance of *Bupalus* and *Ellopia* to be in the ratio of 10:1.

4 Discussion

4.1 Main aspects of the density effect in Bupalus

A summary of the various aspects of the density effect in *Bupalus* gives the following picture.

a. Aggregation reduces growth. Several other factors can also affect growth. In the field, however, the density is much more determinative for average size of individual than abiotic factors. Aggregation does not affect the various external dimensions in a different way; the small individuals resulting from aggregated conditions have the same bodily proportions as the large isolated specimens.

Aggregation reduces fecundity, but not to the same extent that would be expected from the correlation between fecundity and pupal weight. This alleviation of the expected reduction in fecundity has two causes. Firstly, smaller eggs are laid after aggregation. Secondly, egg retention at death is lowest in females originating from crowded conditions.[1]

Mortality is not affected by aggregation, except with severe overcrowding.

The results for viability of the offspring in relation to density conditions in the parental generation are contradictory; on the evidence now available, I think it likely that viability of the progeny is not affected in this way.

Qualitative effects such as changes in pigmentation or larval behaviour, which are associated with shifts in density in certain other species of insect, were not found.

So far, from an ecological point of view, the reduction of fecundity seems to be the most important aspect of the density effect in *Bupalus*.

b. The mechanism underlying the density effect is rather specific. Bodily contacts between larvae, during which regurgitated fluid is transmitted, are necessary for its induction. To be effective, these contacts have to be repeated during several nights.

Since the eggs are laid in groups, distribution of the larvae just after hatching is aggregated, whatever the over-all population density may be. Nevertheless, population density can determine the degree of mutual interference and growth, because aggre-

1. This remark applies to the experimental results. I have no data on egg retention in the field, let alone its correlation with larval density in the previous year. In females found dead in the field, Bevan & Paramonov (1957), Klomp (1966) and Subklew (1939) found negligible numbers of ripe eggs, but in rearing experiments the average retention of eggs was much higher. However, since population densities pertaining to these field data were not given, the results do not help to answer our question.

gation does not affect L1 and the larvae start to disperse immediately after hatching.

The causation of the effect of density is unspecific to the extent that the larvae of at least one other pine-inhabiting geometrid can induce it fully. In the pine forests concerned, however, this species is rare as compared to the average abundance of *Bupalus*.

Thus, the term 'density effect', in the sense of an intraspecific relationship between density and growth and fecundity, is valid in this context. But if the relative abundance of the species concerned differed from that just described, the growth and fecundity of *Bupalus* would probably be determined by density of *Bupalus* and certain other species.

c. The degree of the effect of mutual interference on growth is not constant; rather, there are individual – and probably genetically determined – differences in susceptibility. Consequently, there is a basis for selection against the density effect. Nevertheless, the fact that such an effect has been demonstrated proves that it has not been eliminated by selection. Thus, it must have survival value, and the disadvantage of reduced fecundity must be outweighed.

We are far from having a definite answer to this last, most intriguing, problem, but the literature on similar phenomena in several other insect species as well as some observations in *Bupalus*, can serve as a basis for speculation on this point. This is the theme of the following pages.

4.2 Density effects in insects: their expression, mechanism, and possible function

4.2.1 Expression

Effects of crowding in experiments or of high larval density in the field have been found in several insects, but their expression is as variable as their occurrence is general.

Table 44 gives the data on density effects in Lepidoptera found in the literature; some further points, omitted from this table for the sake of brevity, are mentioned on p. 91.

As appears from Table 44, none of the more frequently studied characteristics is similarly affected by crowding in all species; in fact, every species seems to present a special case. Even within one species, fundamental differences can be observed: between authors' results, between different stages, or between different degrees of crowding (see *Mamestra brassicae*, *Pieris brassicae*, *Spodoptera littoralis*, *Artona funeralis*, *Euproctis chrysorrhoea*, and *Pieris rapae crucivora*).

This is not surprising, in view of the divergent use of the term 'crowding'. In species or stages with gregarious habits crowding represents the natural situation, and in such cases it often promotes survival and growth. Of the numerous published cases of crowding effects in insects with solitary habits, some are certainly attributable to 'overcrowding', but a rigid criterion to distinguish 'overcrowding' from 'crowding'

Additions to Table 44

Activity of the larvae

Crowding induces greater activity in *Leucania separata* (Iwao, 1962), *Plusia gamma* (Long, 1953), *Spodoptera exempta*, and *S. exigua* – but not in *S. abyssinia* – (Faure, 1943a, b), – and in *Exaereta ulmi* (Sharov, 1953). If there is any of such effect in *Bupalus*, it is limited to L1.

Resistance to unfavourable conditions

When larval density is high, larvae of *Leucania separata* accept a greater number of plant species as food and exhibit a greater resistance to starvation than at low density (Iwao, 1962, 1967).

Synchronization of development

Synchronization of larval development is increased by crowding in *Leucania separata* (Iwao, 1962), *Mamestra brassicae* (Hirata, 1956) and *Plusia gamma* (Long, 1953). No such effect has been found in *Bupalus*.

Influence of different degrees of crowding on mortality and growth

Mortality is lowest at moderate degrees of aggregation in *Plusia gamma* and *Diataraxia oleracea* (Long, 1953), and in *Pieris rapae crucivora* (Morimoto, 1960a). Maximum growth is attained at moderate degrees of aggregation in *Pieris brassicae* (Wardziński, 1938) and *Pieris rapae crucivora* (Morimoto, 1960a). In species changing from gregarious to solitary habits during the larval stage, an initially favourable effect of aggregation may become detrimental in the later stages (*Chilo suppressalis*, Morimoto, 1960b, Morimoto & Sato, 1962; *Artona funeralis*, Sugimoto, 1962, Mizuta, 1968; *Euproctis chrysorrhoea*, Grison, 1948).

Chemical composition

Crowding increases fat content in *Plusia gamma* (Long, 1953), *Spodoptera exempta*, and *S. abyssinia* (Matthee, 1945). It decreases fat content in *Agrotis ypsilon* (Zaher & Moussa, 1963).

Diapause

Crowding induces diapause in *Naranga aenescens* (Iwao, 1962) and *Plodia interpunctella* (Tsuji, 1959).

Colour of adults

In contrast to larval colouration, the colour of the adults is hardly ever affected by crowding of the larvae. Only in *Spodoptera exempta* are the imagines from crowded cultures slightly darker than those from single rearings (Faure, 1943b), and in *Lymantria dispar* they are lighter (Leonard, 1968).

Viability of eggs

Hirata (1956) found increased viability of eggs in crowded cultures of *Mamestra brassicae*, but Bonnemaison's data (1962a) do not corroborate this. In *Spodoptera littoralis*, Rivnay & Meisner (1966) observed some decrease in egg viability, of doubtful significance, in crowded cultures.

Elytron femur ratios

In some species, elytron femur ratios increase with crowding. Wing loading, however, provides a more direct approach to the problem concerned.

1. Table 44a contains density effects of the sort referred to on p. 96.
2. See additions on p. 91.
3. (aggr.) = occasionally aggregated.

Columns 6–19: + means that crowding induces a higher rate, value, number; – means the opposite; 0 means that no effect was found.

Table 44a. Density effects in Lepidoptera[1].

	1 Data from	2 Eggs laid (in)	3 Habit of larvae	4 Habit of adults	5 Effect on larvae (in) darker when crowded	6 rate of development	7 number of instars	8 weight and/or size	9 mortality
Geometridae									
Bupalus piniarius	field	groups	solitary		0		–	–	
	exp.				0	–	–	–	0
Ennomos subsignarius	field	masses			+	–			
	exp.								
Noctuidae									
Leucania loreyi	exp.	groups	solitary		+	–			+
Leucania placida	exp.		solitary		0	–		–	+
Leucania separata	field	groups	aggr., later	migrant	+	+		–	
	exp.		solitary or (aggr.)[3]						
Lithacodia stygia	exp.	groups	solitary (aggr.)		+	+			–
Maliattha signifera	exp.	singly	solitary			–			+
Mamestra brassicae	exp.	masses			+	+			
					+	+			
Naranga aenescens	exp.	singly couples	solitary		0	0			
Plusia gamma	field	groups	loose aggr., later	migrant	+	+	–		–[4]
	exp.		solitary						
Spodoptera exempta	exp.	groups	solitary (aggr.)	migrant	+		+	–	
Spodoptera littoralis	exp.	masses	solitary		+	+			0
(=Prodenia litura)	exp.		(aggr.)		+	–			+
	exp.				+	+			
Pieridae									
Pieris brassicae	exp.	groups	aggr., later	migrant	0	+			
			solitary or (aggr.)						
Pieris rapae crucivora	exp.	groups							
intermediate density						+		+	–
high density								–	+
Hesperiidae									
Parnara guttata	exp.	singly	solitary	migrant	+	–			+
Notodontidae									
Exaereta ulmi	field	singly	solitary		+				
	exp.								

10	11	12	13	14	15	16	17	18	19	References
Effect on pupae in			Effect on adults in							
duration pupal stage	weight and/or size	mortality	longevity	preoviposition period	weight and/or size	fecundity	weight of egg	wing area	wing loading	
	–					–				Klomp, 1966
+	–	0	0		–	–	–	–	–	this paper
-	–					–				Drooz, 1966a, b
+	–		0	0		0				Iwao, 1962
	–									Iwao, 1962
-	–		+			0			–	Iwao, 1962
	–									Iwao, 1962
	–									Iwao, 1962
	–					–				Bonnemaison, 1962a
+	–		0			+			–	Hirata, 1956
	–		0	+		–				Iwao, 1962
+	–		+	–	–	+		–	+	Williams & Long, 1950; Long, 1953, 1959; Long & Zaher, 1958; Zaher & Long, 1959
0										Faure, 1943b; Matthee, 1946
0?		0								Iwao, 1962
-?		0	–	–		–				Rivnay & Meisner, 1966
+	–		+		–	0	–			Zaher & Moussa, 1961
-	–		+	+	–	–	–	–	–	David & Gardiner, 1962; Long, 1953, 1959; Long & Zaher, 1958, Wardziński, 1938; Zaher & Long, 1959
0										
0	+									Morimoto, 1960a
	–		–		–					Morimoto, 1960a
			–			–				Iwao, 1962
						+				Sharov, 1953

Table 44b. Density effects in Lepidoptera.

	1 Data from	2 Eggs laid (in)	3 Habit of larvae	4 Habit of adults	5 Effects on larvae (in) darker when crowded	6 rate of development	7 number of instars	8 weight and/or size	9 mortality
Noctuidae									
Agrotis ypsilon	exp.				+	–			
Diataraxia oleracea	exp.	groups	loose aggr.		+	+	–		–[3]
Orthosia cruda	exp.	groups	loose aggr.		+				
Orthosia gothica	exp.	groups	loose aggr.		+				
Orthosia incerta	exp.	groups	loose aggr.		+				
Orthosia stabilis	exp.	groups	loose aggr.		0 to +				
Persectania ewingi	field exp.				+				
Spodoptera abyssinia	exp.				+	0	+?		
Spodoptera exigua	exp.				+	–?			
Trachea artriplis	exp.		solitary (aggr.)		+	+			
Arctiidae									
Arctia caja	exp.					–		–	+
Lymantriidae									
Euproctis chrysorrhoea	exp.	masses	<L2 : aggr. >L2 : solit.			+ 0		+ 0 to +	
Euproctis pseudoconspersa	exp.	masses	aggr.			+	–		–
Lymantria dispar	exp.	masses	solitary			–			
	exp.					0		–	+
	exp.					+	+	–	+
Sphingidae									
Dilana tiliae	exp.	singly-couples	solitary		0				
Laothoe populi	exp.	singly-couples	solitary		0				
Bombycidae									
Bombyx mori	exp.		aggr.					+	
Saturnidae									
Saturnia pavonia	exp.	groups	aggr., later solitary		+	0			
Phyticidae									
Ephestia cautella	exp.					–	+	–	
Crambidae									
Chilo suppressalis	exp.	masses	aggr., later solitary			–		–	[2]
Zygaenidae									
Artona funeralis	exp.	masses	aggr., later solitary			–	+		[2]
	exp.					–	–		[2]

10	11	12	13	14	15	16	17	18	19	References
Effect on pupae in			Effect on adults in							
duration pupal stage	weight and/or size	mortality	longevity	preoviposition period	weight and/or size	fecundity	weight of egg	wing area	wing loading	
0	–			+?		–				Zaher & Moussa, 1963
										Long, 1953
										Long, 1953
										Long, 1953
										Long, 1953
										Long, 1953
										Doull, 1953
										Faure, 1943b
										Matthee, 1947
+?				+?						Faure, 1943a
										Iwao, 1962
					–		0			Hofmann, 1934
										Grison, 1948
										Grison, 1948
	0					0				Mizuta, 1960
+	–					–				Mizuta, 1960
						–				Hofmann, 1934
-	–	+				–				Leonard, 1968
										Long, 1953
										Long, 1953
	+				+	+				Legay & Pascal, 1951
										Long, 1953
					–	–				Takahashi, 1961a, b, c
0	–	+			–	–				Morimoto, 1960b
										Sugimoto, 1962
0	–					–				Mizuta, 1968

is difficult to give. Furthermore, crowding may refer both to a situation prevailing more or less frequently in nature and to one occurring only in the laboratory. As to their mechanism, some crowding effects are clearly due to competition and others to mutual interference; and in several instances the possible causes have not been disentangled.

Grassé (1958) has made a distinction between 'group' and 'mass' effects. In short, the former are caused by direct sensory stimulation between individuals of the same species and the latter are due to a modification of the environment by the aggregation. Grassé's classification is based on only a single aspect of the complex biological phemomena involved, namely the physiological mechanism, and this, in my opinion, makes its usefulness questionable.

In the case of *Bupalus*, we are concerned with *mutual interference* between larvae with solitary habits, resulting in a *chronic change* in certain characters and occurring at densities commonly found *in natural populations*. It is to this form of density effect that the discussion in the following pages will be limited. Neither competition, the effects of which are immediate and often readily reversible (at least in an early stage), nor caste polymorphism of social insects will be considered.

An attempt to select from Table 44 the cases belonging to this class, encounters another difficulty. Comparability with conditions in the field should be judged on the basis of knowledge of the causative mechanism underlying the density effect: what is relevant is not the number of individuals per unit volume but the amount of effective stimulus received by the average individual. Excessive prudence, however, would prohibit any choice, since, as we shall see, the precise nature of the stimulus involved is insufficiently known in almost every case.

The cases of probable relevance among the Lepidoptera have been collected in the first part (a) of Table 44. Instances of this sort of density effect in other groups of insects have been summarized at the end of this section.

Despite the wide variation, there is an undeniable similarity in the effect of density (in the more limited sense) on some characteristics of Lepidoptera:

a. When a change in larval colouration is involved, crowding produces darker and more contrastingly coloured larvae, whereas the solitary type is more cryptically coloured. Unlike the situation in locusts, no corresponding changes in adult colouration have been observed (except in two cases), which is understandable because of the difference in habits between larvae and adults in Holometabola (Iwao, 1962; Long, 1953).

b. In several species activity, or immediate behavioural response to certain stimuli, is more intense in crowded larvae. In these cases, solitary larvae show skulking behaviour and, unlike crowded ones, often feed at night.

c. Individual weight is frequently affected, crowding almost always causing a decrease. Frequently, though not invariably, is a decrease in fecundity associated with this.

d. Where studied, wing loading is slightly decreased by crowding. This relation has received special attention in Lepidoptera because some species exhibiting a density

effect are known or supposed to be migratory, and because of the similarity with phase phenomena in locusts (Brown, 1962; Iwao, 1962; Long, 1959).

The same points hold more or less for density effects in the other groups of insects. Wing dimorphism is frequently involved, brachypterism invariably being associated with low densities and macropterism with high ones.

A summary of density effects in non-lepidopterous species

Orthoptera In locusts *(Locusta migratoria* subspp., *Schistocerca gregaria, Nomadacris septemfasciata, Dociostaurus maroccanus, Locustana pardalina, Chortoicetes terminifera)* crowding affects behaviour, colour, morphology and physiology. Some effects are partially transmitted to the next generation(s), which gives rise to a continuous polymorphic series. The effects vary with species (for details see Albrecht, 1967; Uvarov, 1966). According to current opinion, phase polymorphism enables these species to thrive in heterogeneous and often rapidly changing environments, each of the phases being adapted to one of the two possible extremes (Kennedy, 1956, 1962). Similar variations associated with density have been found in certain non-swarming grasshoppers, e.g. *Chorthippus albomarginatus* (Rubtzov, 1935).

In *Acheta domesticus,* Chauvin (1958) and McFarlane (1962) found faster growth and higher adult weight of grouped against single larvae; Chauvin's data suggest optimum growth with moderate crowding. Fuzeau-Braesch (1960, 1962) found dark pigmentation in isolated *Gryllus bimaculatus* and a light colour after grouping of two or more larvae.

Phasmidae Key (1957) reports an effect of density in the field and in cultures on colour and morphometric characters of *Podacanthus wilkinsonii,* and to a lesser extent in *Didymuria violescens* and *Ctenomorphodes tessulata.* As in locust hoppers and in *Locusta* adults, green predominated at low, a yellow and black colour pattern at high density.

Kirchner (1939) found decreased fecundity with increased larval density in experiments with *Carausius morosus.*

Dermaptera In Lhoste's (1944) trials, grouped larvae of *Forficula auricularia* consumed less oxygen and gave smaller adults than isolated specimens.

Dictyoptera In experiments with *Periplaneta orientalis,* larval crowding increased larval mortality and rate of development, and gave smaller adults (Landowski, 1938).

Psocoptera Larval crowding in experiments with *Psyllipsocus ramburi* gave macropterous females, and isolation gave micropterous or brachypterous individuals (Badonnel, 1948, 1949).

Hemiptera Johno (1963), Kisimoto (1956), and Watanabe (1967) found larval density to be a factor influencing wing form in *Nilaparvata lugens* and *Laodelphax striatellus.* Low density increases the proportion of brachypterous individuals, but a different reaction of the sexes complicates the situation.

In experiments with *Nezara viridula,* high larval density darkened L4 and L5 and reduced weight and fecundity of adults (Kiritani, 1964; Kiritani & Kimura, 1965).

In *Eurydema rugosum,* the larvae of which live in groups, highest adult weight, fastest development, and lowest mortality were obtained at medium experimental densities (Kiritani & Kimura, 1965).

Youdeowei (1967) found a better synchronization of moults, lower mortality, and reduced adult weight at the higher larval densities in experiments with *Dysdercus intermedius.*

In aphids, crowding of larvae or viviparous adults (depending on species) induces development of wings or of winged progeny (Bonnemaison, 1951, 1959: *Brevicoryne brassicae, Myzus persicae, Dysaphis plantaginea;* Johnson, 1965: *Aphis craccivora;* Lees, 1966: *Megoura viciae;* Toba

et al., 1967: *Therioaphis maculata)*. The influence of other factors may complicate this relation (see Hille Ris Lambers, 1966).

Coleoptera In *Callosobruchus maculatus*, larval crowding induces the active flying form, whereas inactive adults are produced at low densities (Sano, 1967; Utida, 1956).

Contamination of the medium, resulting from larval or adult crowding, prolonged larval development and reduced adult weight in experiments with *Tribolium castaneum* and *T. confusum* (Karten, 1965; Park, 1941). Similar effects were found in *Ptinus tectus* (Gunn & Knight, 1945). Crowding delays pupation in *Trygoderma granarium* (Stanic *et al.*, 1963).

In experiments with *Cryptolestes turcicus*, the rate of development, survival, and adult weight decreased with increasing density. Contamination of the food as well as mutual interference were involved in the causation of these effects (Lefkovitch, 1962).

4.2.2 Mechanism

Stimuli involved The stimulus or stimuli operating in density effects have proven difficult to identify. In Lepidoptera, experiments on this point have been performed in *Mamestra brassicae* (Bonnemaison, 1962b; Hirata, 1956), *Ennomos subsignarius* (Drooz, 1966b), *Leucania separata*, and *Naranga aenescens* (Iwao, 1962), *Plusia gamma* (Long, 1953, 1955), *Exaereta ulmi* (Sharov, 1953), and *Ephestia cautella* (Takahashi, 1961c). Of these authors, only Sharov expresses a definite opinion about the factor involved, namely aspecific mechanical stimulation.

Visual stimuli were found to be ineffective in Bonnemaison's and Long's experiments as well as those of Johno (1963) with the bug, *Nilaparvata lugens*, Johnson (1965) and Lees (1966, 1967) with the aphids, *Aphis craccivora* and *Megoura viciae*, and Badonnel (1949) with the Psocopteron, *Psyllipsocus ramburi*. On the other hand, visual stimuli are involved to some extent in the induction of certain phase characteristics of locusts (Chauvin, 1941; Ellis, 1962), and in the density effect in *Gryllus bimaculatus* (Levita, 1962).

Olfactory stimulation proved ineffective in the experiments of Drooz, Iwao, Lees, Long and Toba *et al.* (1967). Bonnemaison found only a slight effect of odour. Badonnel obtained some evidence for olfactory stimulation in *Psyllipsocus ramburi*, and Levita for olfactory or vibratory stimuli in *Gryllus bimaculatus*.

Contamination of the food was found ineffective in *Leucania separata* and *Naranga aenescens* (Iwao), *Ennomos subsignarius* (Drooz) and in *Therioaphis maculata* (Toba *et al.*). Although recent studies have shown that some form of mutual interference is responsible for the induction of wing development in two species of aphid, the possibility of morph induction in other species under the influence of crowding via the food plant, has not been disproved. It is in Heteroptera that this kind of stimulus may be expected (see Nuorteva, 1958).

Contamination of the medium with excreta is one of the factors inducing the density effect in *Ephestia cautella* in rice bran (the others being contact stimulation and, at higher densities, competition for food; Takahashi, 1961c). In stored-product beetles, contamination has repeatedly been found to be the cause or one of the causes of crowding phenomena: in *Tribolium castaneum* and *T. confusum*, by ethyl-

quinone, a gaseous excretory product (Alexander & Barton, 1943; Karten, 1965; Park, 1941; Roth, 1943), in *Ptinus tectus* (Gunn & Knight, 1945), in *Cryptolestes turcicus* (Lefkovitch, 1962), and in *Trogoderma granarium* through accumulation of faeces (Stanic *et al.*, 1963).

In *Callosobruchus maculatus*, crowding takes effect through local increase of temperature (Sano, 1967).

Experimental proof that CO_2 accumulation is not the causative factor in the insects concerned has been given by Bonnemaison (1962b), Drooz, Iwao; Levita, and Long (1955).

It has often been suggested (generally after the elimination of other possibilities) that physical contacts must play an important part in the mechanism of density effects. The precise nature of these contacts is not yet known; in particular, information on the possible transmission of more or less specific chemical substances during contacts is very scanty.

In Lepidoptera, the contact stimuli are not (Sharov, 1953) or not completely (Drooz, 1966b; Iwao, 1962) specific. Long (1953, 1955), on the other hand, found no effect of the presence of *Pieris brassicae* or *Diataraxia oleracea* on *Plusia gamma*. Long observed vehement movements of contacting larvae and sometimes regurgitation, but whether this has anything to do with the stimulus is not known.

Tactile stimulation is the principal factor inducing the density effect in *Gryllus bimaculatus;* some related species of *Gryllus* are equally or almost as effective in inducing the colour changes as *G. bimaculatus* itself, but one less closely related *Gryllus* species is virtually ineffective (Levita, 1962). In *Locusta migratoria migratorioides*, certain behavioural characters of the gregarious phase are induced by unspecific tactile stimuli (Ellis, 1962). Chauvin (1941) concludes that visual and tactile stimuli together induce the gregarious colouration in *Schistocerca gregaria;* in view of the results of his experiments on grouping of *Schistocerca* with other species, the relevant stimuli must have a certain specificity. Some acridologists feel that the possible role of chemical factors is not yet sufficiently eliminated (e.g. Albrecht, 1967, p. 130). Indeed, Nolte (1963) published some evidence for a pheromone inducing melanization in three species of locust. Sexual maturation in immature *Schistocerca* adults is stimulated by the presence of mature males (Norris, 1954). The mechanism of this is a pheromone produced in the epidermal cells and transferred during contacts, or less efficiently, perceived through olfaction at short distances (Loher, 1961). Furthermore, ovipositing *Schistocerca* females tend to seek each other's presence, and the mechanism underlying this behaviour has a strong chemical component (Norris, 1963). Gillett (1968) found evidence for an airborne factor in the induction of gregarious characteristics in *Schistocerca gregaria.*

Johnson (1965), Lees (1966, 1967), and Toba *et al.* (1967) conclude that unspecific tactile stimuli are the principal or sole factor involved in density-induced production of alatae in the aphids *Aphis craccivora*, *Megoura viciae*, and *Therioaphis maculata.* In *Megoura* contact transmission of a pheromone does not occur (Lees, 1967).

Our results show that in *Bupalus*, direct contacts are essential for the full response.

Mechanical stimuli alone are, however, ineffective; the contact transmission of a chemical factor, which apparently is contained in regurgitated gut fluid, is necessary. The indirect transmission of this factor is at best very limited and without any significance under natural conditions. As has already been pointed out (p. 84), this conclusion remains subject to some reservation, because artificial stimulation with regurgitated gut fluid failed to give the usual durability of the density effect.

It is rather puzzling that the larvae apparently are insensitive to their own fluid. If they were sensitive, mechanical stimulation (which makes the larvae regurgitate) could hardly remain without effect as, in fact, it did. Or perhaps is the effective substance emitted in such a way that the chance of its perception by the emitting larva is very small, as Eisner *et al.* (1963) have suggested with respect to the defensive secretion of the beetle *Calosoma promineus*. These points remain open to more detailed experimentation.

In addition to a fundamental difference in response to density between the instars (L1 being unsusceptible), there is evidence for a daily rhythm in this respect. The best indication for this is that artifical encounters between larvae arranged at some time in the photophase were totally ineffective. This may be due to a rhythm of susceptibility or (and) to a rhythm in stimulative ability; I have not gone into this further. Circadian rhythms in responsiveness of males to sex pheromones have been found in a number of noctuids (Shorey & Gaston, 1965).

. *Perception of stimuli* In the perception of tactile stimuli inducing gregarious behaviour in *Locusta*, the antennae play an important part (Ellis, 1962). Loher (1961) obtained similar results with regard to the perception of the maturation-accelerating pheromone in *Schistocerca*. Chauvin (1958) claims to have shown that the antennae and the cerci are involved in the effect of grouping in *Acheta domesticus*, but Chauvin's interpretation of his amputation experiments is questionable (see also McFarlane, 1962, and Rummel, 1963). The tactile stimuli in *Gryllus bimaculatus* referred to above are perceived by the antennae (Levita, 1962).

In the aphids *Aphis craccivora* and *Megoura viciae*, the antennae are not essential for the perception of the stimuli; but in *Brevicoryne brassicae* their amputation diminishes the response to crowding (Bonnemaison, 1951).

The site of perception of the density stimuli in *Bupalus* has not been investigated.

Physiological processes controlling density effects For locusts, abundant evidence has been accumulated regarding the hormonal control of the behavioural, chromatic, and reproductive aspects of the phases, although all the details of the processes involved are far from being known (see Uvarov, 1966, for synopsis; Cassier, 1965). Uvarov concludes that 'phase polymorphism in all its aspects will ultimately be found to depend on differences in the physiology of the sensory, nervous and endocrine systems'.

Wing dimorphism in aphids in under endocrine control, and the juvenile hormone plays a dominant role (Lees, 1966). Hardly any work has been done in this respect

on Lepidoptera. Morimoto (1960a) found the corpora allata to be larger in crowded *Pieris rapae* than in single larvae, but this case is too isolated to permit interpretation or comparison.

It is clear that the growth reaction to aggregation in *Bupalus* is not simply the result of disturbed feeding; more fundamental physiological processes must be primarily affected (Chapter 2, p. 47-53; Chapter 3, p. 78). Endocrine changes are undoubtedly involved, but any speculation about their possible nature would, in the present state of our knowledge, be premature.

4.2.3 Possible function

Attempts to hypothesize on the possible function(s) of density effects should start from the basic assumption that natural selection favours genotypes that are able to survive and to produce the greatest number of viable offspring.

Besides its dependence on intrinsic qualities, the survival of parents and offspring is determined by environmental conditions, among which the density of the population to which they belong is one of the factors. Therefore, genotypic ability of an individual to respond in some way or other to changes in population density can have survival value for itself or for its progeny, and thus give a selective advantage. The interesting question is: what relationships are involved in the various cases? Some of the possibilities warrant discussion.

a. Firstly, high density can be deleterious owing to depletion of certain resources; and either some form of mutual interference or actual competition may induce an escape reaction, e.g. dispersal or diapause. In the former case (representing a density effect in the sense referred to on p. 96), mutual interference is the proximate factor inducing dispersal, and competition the ultimate one; and in the latter case, the proximate factor and the ultimate one are the same. It is not always easy to distinguish which of the two mechanisms is involved in a given case.

Iwao (1962) has interpreted the migratory tendency of the larvae of the army worm, *Leucania separata*, and the greater number of plant species they accept as food under crowded conditions, in this sense. There is some evidence suggesting similar relationships in other species of army worm, but the experimental data are not yet sufficiently extensive to be convincing (see review in Brown, 1962). According to Quo *et al.* (1963), the adults of *Leucania separata* migrate to a habitat more suitable for themselves and their offspring when the larvae or pupae from which they have issued had been subjected to unfavourable conditions.

High larval density in the meal moth, *Plodia interpunctella*, induces diapause (Tsuji, 1959).

Dempster (1968) describes a nice example of dispersal induced by competition in a field population of a psyllid on broom.

b. A second possibility, more likely to occur in field populations than the preceding one, is a reaction to escape density-related mortality arising from natural enemies. In this case, a reaction can already be expected at relatively low population densities.

Here, a further distinction can be made: between a reaction to escape mortality in the same generation, and one to avoid high mortality among the progeny.

Exaereta ulmi, studied by Sharov (1953), presents the best example of the first type. At low population density in the field, two types of procryptically coloured larvae are found, one matching the leaves, and the other twigs of the food plant (elm trees). The larvae feed at night, and they cling to the substrate when touched or when the tree is shaken. At high population density, colour and behaviour of the later larval instars are altered profoundly: colouration is contrasting, with melanized patches; the larvae feed during the day and drop as soon as the trees are shaken. Sharov presents evidence for the survival value of the density-induced types as a defense against predation by birds.

Key (1957) has suggested a similar function of phase colouration in some phasmid species ('facultative aposematism') and Lea (1962) for *Locustana pardalina*. But no work has been done to test the validity of this hypothesis.

It is tempting to recognize the second type in the army worms, *Leucania separata* (Iwao, 1962) and *Spodoptera exempta* (Brown, 1962). Both species exhibit sudden outbreaks, which seldom last for longer than one generation. It is not yet certain whether these outbreaks, in Spodoptera exempta, originate from sparse autochthonous populations, from migrating adults, or from both (Brown, 1962; Whellan, 1954, 1958). Whatever the case may be, there is convincing evidence for migratory behaviour of adults in both species (Brown & Swaine 1966; Iwao, 1962; Lin & Chang, 1964). In S. exempta, parasitism is usually low in the first generation, but in the second one in the same place it is usually heavy, and the host may even be wiped out completely. On this basis it has been suggested that adult migration may be functional in avoiding eradication of the progeny by natural enemies (Whellan, 1954); and the influence of crowding on wing loading or flight activity indicates that one of the effects of high density might be to facilitate flight or to induce the tendency to migrate (Brown, 1962; Iwao, 1962). Some further aspects of this point will be considered below.

c. The third possibility is that population density only warns against approaching physical unfavourableness without itself having anything to do with the unfavourable conditions. This case is exemplified by Kennedy's (1956) explanation of the phase phenomenon in locusts (see also Southwood's (1962) extensive discussion of migration of arthropods in relation to physical environmental unsuitability and temporary habitats). The environment of locusts, Kennedy argues, is typically unstable, and consists of an ever-changing pattern of places in an unsuitable area that are temporarily suitable for the solitary phase. When becoming unfavourable, the surface of a suitable place gradually diminishes and the associated local increase of density initiates the shift from the solitary to the gregarious phase. The latter, because of its mode of living, can exploit the 'unfavourable' gregarious habitat.

Other mechanisms than those mentioned are undoubtedly conceivable. The mechanisms suggested above, while applying to a given species, might fail to explain

some of the density-induced changes in that species. This could mean that several functional mechanisms are involved in one species (as, in fact, is suggested above for L. separata). But is also plausible that some of the effects of crowding are afunctional: they would merely represent the relatively small price at which a greater gain is purchased. This might apply to the frequently observed reduction of fecundity under crowded conditions; we will return to this point when we consider the case of *Bupalus*.

In discussing the significance of density effects, several authors (Brown, 1962; Long, 1953; Zaher & Moussa, 1961) have attempted to show that crowding increases the ability of the members of the population to enhance population density. This reasoning confuses the cause of a phenomenon with its function. The crucial point is not the ability to contribute to further density increase, but rather the ability to maximize the chance of survival as well as of effective reproduction, under the changed environmental conditions (*viz.*, high instead of low density).

As to the function of the density effect in *Bupalus*, Klomp (1966) has suggested an explanation of what might be called Sharov's *Exaereta ulmi*-type (p. 102). He draws attention to the procryptic colour of the larvae and their inconspicuous behaviour (both of which are efficient as a defense against predators only in case of dispersed occurrence), and to the seemingly contradictory fact that the eggs are laid in batches.

The function of the density effect is, Klomp supposes, to stimulate dispersal of the larvae from the initial groups to avoid excessive predation. However, for such dispersal to be effective, population density must not surpass a certain level. This leads Klomp to remark finally – unfortunately without further explanation – that 'it is therefore striking that the mechanism of dispersal seems to be so closely bound up with the mechanism regulating population density' (namely reduction of fecundity and possibly of viability of the offspring at high population density). Since this was written, further experimentation has been unable to confirm the initial evidence of an influence of parental density on viability of progeny. We will leave this doubtful effect on viability out of consideration: which only affects Klomp's hypothesis to a degree.

To judge the validity of this hypothesis, answers to the following three questions are relevant:

a. Could the ability of a dispersive reaction of the larvae to encounters be advantageous for survival?

b. Would reduction of growth and fecundity be a physiologically necessary associate of the supposed behavioural (dispersive) reaction to encounters?

The answer to the first question must be given in the affirmative; the answer to the second one in the negative, on the basis of the following argument.

In *Bupalus*, behavioural reactions to encounters occur in L1, without associated growth reactions. In other species in which mutual interference has certain effects, it does not necessarily reduce fecundity (see Table 44). When two larvae of *Adoxophyes reticulana* are reared together in a small tube, one is 'chased from the food' and remains small. When this small individual is separated from the aggressor and reared singly, it eventually makes up its initial arrears in size (van der Meer, unpublished

report). Therefore, behavioural and permanent growth reactions are not necessarily inseparable, and consequently, genotypes exhibiting the (hypothetical) dispersive but not the growth reaction could conceivably occur in *Bupalus*.

c. Would the ability to reduce fecundity under high density be advantageous as compared to the ability to keep fecundity unchanged?

The answer is in the negative. If 'group selection' is accepted (Wynne–Edwards, 1962), the answer to this question could be yes; but Klomp squarely rejects this type of selection. Indeed, in view of the restrictions to which its occurrence is subject (Smith, 1964), it does not provide a very solid basis for functional hypotheses.

Consequently, if Klomp's explanation were valid or exhaustive, I believe that the density effect would have to stimulate larval dispersal (for which there is, in fact, at best only scanty evidence[1]) and leave growth and fecundity unchanged. In reality, however, growth and fecundity are changed, and even by means of a rather specific mechanism. For any hypothesis about function, this fact must be taken into consideration.

Klomp's starting point, that high local density of larvae is disadvantageous owing to an increased chance of mortality arising from natural enemies, is very valuable. With increasing population density in *Bupalus*, the chance of survival of the average individual will decrease, owing to vertebrate predators (for which there is circumstantial evidence; Klomp, 1966) or to entomophagous insects (which is hypothetical), or to both. Female moths could increase their effective reproduction by moving from their place of birth to more scarcely populated areas, where the pressure of predation will be less.

Therefore, I suggest as a hypothesis for further work that high larval density in *Bupalus* leads to an increased tendency of the female moths to disperse, and that density-induced reduction of weight contributes to the realization of this tendency as well as possibly being one of the causes of its intensification (see p. 106). Decreased weight also entails a reduction of the egg-laying capacity, but the greater chance of survival of the offspring after dispersal would enhance effective reproduction. Thus, the explanation suggested here is of the adult dispersal or 'army worm' type.

I have done some work to test this hypothesis, but the results are not conclusive. In the following, I shall first discuss the evidence provided by *Bupalus* in support of this hypothesis and then give some general remarks.

4.3 Adult dispersal in Bupalus

Bupalus is not known to be migratory. In fact, the females are reported to be poor fliers (Engel, 1939), and when flight of moths is observed in the field during the day,

1. Namely in L1, when crowded larvae are possibly more active than solitary ones (Table 27), and when mutual interference does not affect growth. But the need to invoke density-induced dispersal is doubtful, since random dispersal seems adequate to explain the distribution of the larvae at the end of L1.

the males are commonly found to greatly outnumber the females. On the other hand, catches of particularly females in light traps outside the habitat have been reported more than occassionally.[1]

Through the courtesy of lepidopterologists operating light traps, I obtained additional data on *Bupalus* catches in and at a distance from Scots pine forests (Table 45).[2] The catches mentioned for 1966 were made in three widely separated places: near Arnhem, near Diepenveen (37 km NE of Arnhem), and near Stein (130 km S of Arnhem).

The striking point in this material is in the differences in sex ratio, which seem to be related to the position of the traps in relation to the nearest pine forest. In the forest, males predominate in the catch, and outside it, females; at the forest's edge, the catch is evenly distributed over the sexes. The results of 1967, when a forest trap was added in Diepenveen, corroborate these findings.

Since Klomp (1966) found the sex ratio at emergence of the moths to be close to unity, it can be concluded that flight activity is greater in males than in females (catches of forest traps), and that the tendency to leave the forest is definitely greater in females than in males. Females caught outside the forest still contain part of their initial egg supply (Table 45).

It is of interest to compare these results with Klomp's (1966) description of the behaviour of ovipositing females: 'the eggs are deposited on the needles in clusters of 2 to 25. After each act of oviposition the moth has a period of rest, and – before resuming the laying act again – a short flight is performed as a result of which the nearly 180 eggs of one female are more or less randomly distributed on the crowns of several trees'. Now, the following assumption about the behaviour of the moths in dependence on larval population density presents itself. Shortly after emergence, the moths copulate in the forest. The females then either remain close to the place in which they hatched and thus deposit their whole egg supply in some limited area (low density situation) or, before each oviposition act, engage in small scale dispersive flights, in which they may leave the forest of birth and move, step by step, over several kilometres (high density situation).

From a behavioural point of view, Klomp's description of the females' habits suggests two opposite, and alternating patterns of behaviour: flight and oviposition.

1. Catches of *Bupalus piniarius*, in light traps or otherwise, outside the habitat, and in some cases at considerable distance from it, have been reported by Boer Leffef (1963), van de Bund (1956), van Daele & Pelerents (1966), Eckstein (1923), de Fluiter *et al.* (1963), Kuchlein (personal communication), Lempke (1952), Nitsche (quoted by Escherich, 1931), and Peerdeman (1965). Where the sex of these moths has been stated, females invariably predominate. Boer Leffef, for instance, mentions a ratio of ♂ : ♀ of 1 : 6 which, he suggests, might be due to the occasional occurrence in females of a tendency to undirected wandering flights.

2. I wish to thank the Rev. Fathers A. Munsters, Stein, and A. Alma, Diepenveen, and Mr G. J. Flint, Deventer, for kindly sending me the *Bupalus* caught in the light traps under their supervision; and Mr I. A. Houck, Almelo, and the Rector of 'Johannahoeve', Oosterbeek, for kind permission to operate light traps on their grounds.

Under the present hypothesis, the threshold between oviposition and flight would be passed more readily (i.e. after only a short flight) by low-density adults than by high-density ones. Reduced mechanical hampering of flight in the latter type, owing to reduced wing loading, could accentuate, if not account for, this difference in threshold (see Kennedy, 1961).

Several authors, notably Johnson (1963, 1966), have discussed insect migration on the basis of the view that most of it is adaptive. In Johnson's conception 'migration' and 'flight adapted for dispersal' are synonymous. In the present discussion, the terms migration and dispersal are used in this sense.

In migratory species dispersal often occurs in the pre-reproductive stage of adult females, but in some species it is interreproductive, i.e. bouts of flight and oviposition alternate (Johnson, 1963).

Table 45. Catches of *Bupalus* moths in light traps operated throughout the flight periods in 1966 and 1967.

	1966				1967			
	number caught		sign. of difference from 1 : 1 sex ratio	number of ripe eggs when caught mean ± SD	number caught		sign. of difference from 1 : 1 sex ratio	number of ripe eggs when caught mean ± SD
	♂	♀			♂	♀		
Arnhem:								
in pine forest	54	13	P<0.01	32 ± 22	[2]			
Diepenveen:								
in pine forest	[2]				13	1	P=0.003	49
edge of pine forest	5	5		47 ± 43	1	2		83 (96; 69) [1]
orchard	1	5	P=0.22	50 ± 12	1	5	P=0.22	38 ± 10
Stein:			0.02					
orchard	3	10	P=0.097	56 ± 18	0	0		

1. Individual data.
2. No trap operated.
Rough estimate of number of eggs in newly emerged females: 180.
Significance of differences: binomial test.
The traps were of the modified Robinson type (van de Pol, 1956), with 125 watt mercury vapour bulbs. They were operated the whole night during the flight period of *Bupalus*.
In Diepenveen the orchard trap was located at a distance of about 150 m from the pine forest as well as from the trap at the edge of the forest, the two being separated by a farmyard, a beech lane, and a wooden fence around the orchard. The forest trap was at a distance of about 150 m from the edge of the forest, the three traps being in a straight line.
The orchard in Stein is situated in the Meuse valley, in park-like surroundings with deciduous and a few coniferous trees but without any Scots pine. The nearest pine forests are about 5 km to the West and about 15 km to the East.

4.3.1 Adult dispersal and larval population density

The crucial point with respect to the hypothesis presented above, is whether dispersal of females is related to larval population density.

A release-recapture experiment with moths from single and aggregated rearings failed to produce evidence on this point. Marked moths of the two density categories were released during the day at a distance of 20 m from a light trap in the forest. A second identical trap was placed in a pasture at about 100 m from the first one and 80 m from the pine forest; a 40 m wide strip of dense coppice separated the pasture from the forest. This trap, unfortunately, failed to catch any *Bupalus*, either marked or wild. This failure contrasts surprisingly with the relatively abundant catches of *Bupalus* outside the habitat shown in Table 45. A possible explanation is that *Bupalus* flying outside the forest are attracted to woody vegetation.

Eckstein (1923), referring to a German Civil Service report dating from 1898, mentions a case of an exodus of *Bupalus* females from forests that had been defoliated by the pine looper in the previous year. Dispersal was observed from forests that had failed to produce a new crop of needles as well as from those bearing new foliage, and the moths flew further than would have been necessary to find green forests. Great numbers of moths were observed in exposed forest edges and in isolated woods (whereas in normal years the moths preferentially stay in the sheltered interior of the forests); moreover, flocks of moths made up predominantly of females, were observed around lights in a nearby city. In normal years, Eckstein remarks, the moths stay in the forests. Nevertheless, some doubt may remain as to whether this kind of dispersal is not merely an annual phenomenon that is observed more readily when the moths are unusually numerous.

Klomp (1966) believes that dispersal of moths from their hatching sites is absent or unimportant. He provides indices of moth 'mortality' which might, however, be interpreted as evidence for density-related dispersal. The index is the quotient of actual and potential egg density, the latter being estimated as density of emerging moths multiplied by their average fecundity. This 'mortality' index may represent mortality, or dispersal, or both. When plotted against density of the larvae from which the moths have issued, an undeniable indication of a positive correlation is obtained (Fig. 16b). For comparison, Klomp's (1966) data on larval density and female pupal size are given in Fig. 16a. Both correlations strikingly seem to level off at about the same larval density of between 10 and 15 larvae per m^2. This favours the supposition that the 'mortality' index represents dispersal associated with moth size (see Fig. 17), rather than moth mortality.

These points form the only, and admittedly far too scanty, direct evidence that can be presented in favour of density-related dispersal of females in *Bupalus*.

In locusts (Kennedy, 1961; Uvarov, 1966) and in several species of aphid (Hille Ris Lambers, 1966; Lees, 1966) crowding induces migration. High larval density in the beetle *Callosobruchus maculatus* induces active, flying adults, whereas adults from low density conditons are incapable of flight (Sano, 1967; Utida, 1956). In

Fig. 16. Relation between larval population density and female pupal diameter in the following winter (a), and subsequent mortality of female moths (b), in the field population studied by Klomp (1966).

In graph b, the point in the lower right corner is out of line. When it is included, Kendall's correlation coefficient $\tau = 0.29$ ($P = 0.16$). When it is excluded (there is no reason to do so apart from its aberrant position), $\tau = 0.45$ ($P = 0.03$).

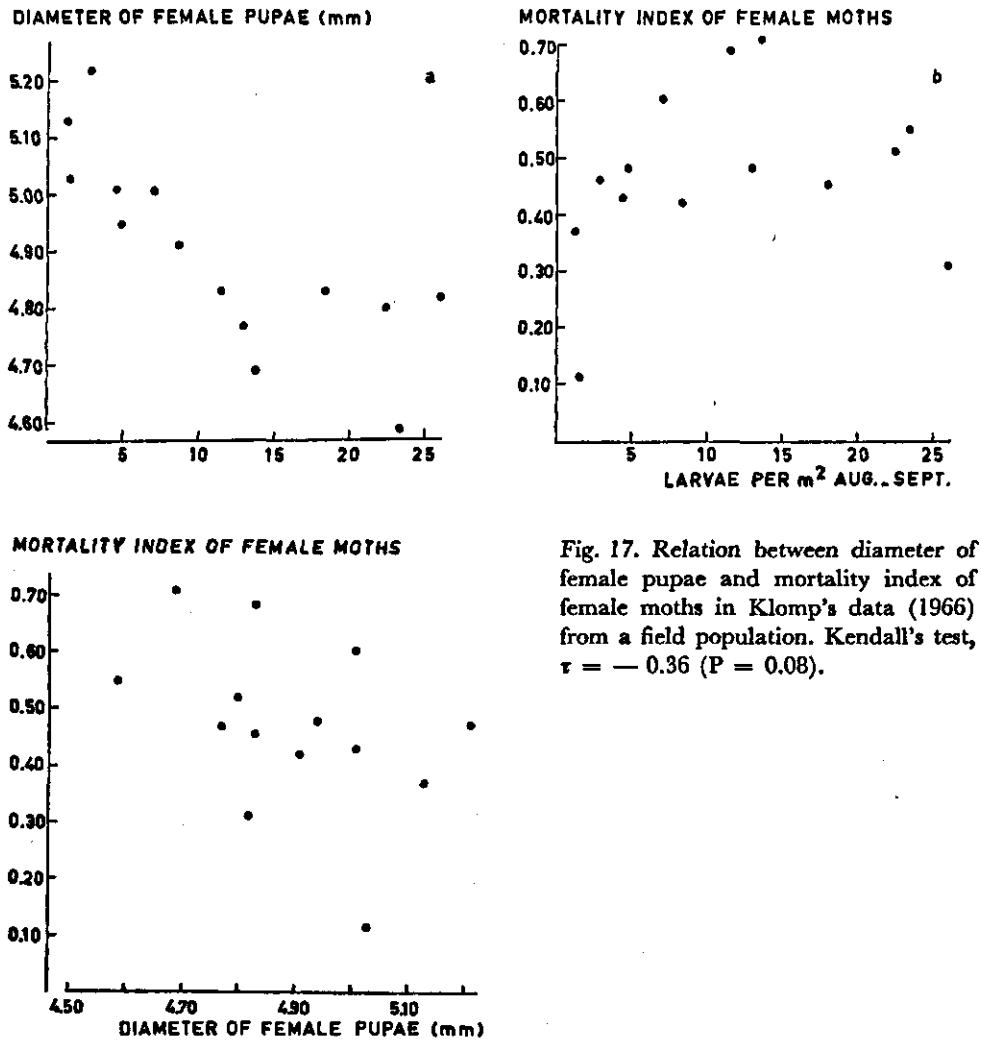


Fig. 17. Relation between diameter of female pupae and mortality index of female moths in Klomp's data (1966) from a field population. Kendall's test, $\tau = -0.36$ ($P = 0.08$).

Lepidoptera, a relation between larval density and adult dispersal, owing to decreased quantity and quality of larval food, has been found in *Choristoneura fumiferana;* dispersive ability being associated with reduced egg-laying capacity (Blais, 1953; Greenbank, 1963; Miller, 1963). In *Mamestra brassicae,* larval crowding enhances flying activity of the adults (Hirata, 1956). There are also some other instances in which density-related dispersal has been suggested in Lepidoptera, the

mechanisms involved being unknown (Burmann, 1965: *Zeiraphera diniana;* Nielsen & Nielsen, 1950: *Ascia monuste;* Wellenstein, 1942: *Lymantria monacha*). Paradis & Le-Roux (1965) studied the population dynamics of the tortricid *Archips argyrospilus* in an unsprayed apple orchard over a period of 7 years covering an outbreak cycle. They found immigration of moths in the progression years and emigration in the years of population maximum and decline, but heavy spraying in the surrounding orchards during the outbreak made it impossible to decide whether migration was density related. Moreover, this point was not given special investigation. It was clearly shown, however, that bird predation of larvae and pupae was an important density-dependent mortality factor. These data are suggestive of a possible relation between population density, predation, and density-induced dispersal of adults.

4.3.2 Wing loading

As we have seen, density conditions induce changes in morphological characters associated with flight in several species. In Lepidoptera, a greater wing area relative to body weight as a result of larval crowding has been found repeatedly (Table 44). Long (1959) has extensively discussed this relationship in *Plusia gamma* and *Pieris brassicae*. A similar reduction of wing loading associated with crowding was found in *Bupalus* females (Table 29).

Although wing loading certainly plays an important part in flight, the significance of relatively small variations in its value are difficult to assess. The power required to raise an insect from the ground increases as the 3.5th power of a linear dimension, and the wing area as the square (Dorsett, 1962). Dorsett found that within one species of Spingid moth the intra-thoracic temperature, and probably the wing-beat frequency required for take-off, increases with wing loading.

4.3.3 Adult dispersal and net reproduction

If adult dispersal is to be an efficient mechanism for the protection of the progeny against density-related action of natural enemies, dispersing adults must have a fair chance of reaching sparsely populated pine forests. Although some short-term studies have been made to compare population densities of *Bupalus* in different parts of a forest area, more extensive comparative data on population fluctuations are not available.

In years of high over-all abundance, Bevan (1961), Davies (1962), and Engel (1942) found considerable differences in the population density of different compartments of the forests they investigated (involving factors of 10 to 300 and more). Within a distance of several kilometres, local density could vary from extremely low to very high. Engel, comparing three compartments differing in forest composition but located in the same area, found a relatively high generation survival in the sparsely populated mixed forest compartments and a relatively low one in an even-aged stand with high *Bupalus* density.

Table 46. Number of larvae descending from the tree crowns in autumn, per m².

	Plijmen (Klomp, 1966, and personal communication)	Oldebroek
1963	8.6	8.6
1964	1.6	3.9
1965	0.8	1.0
1966	0.2	2.6
1967	0.1	3.3

Klomp's (1966) data concerning a homogeneous 7-hectare area of a pine forest ('Plijmen') revealed no heterogeneity in population density other than that ascribable to random variation. But if other stands were to be sampled, real differences would probably be found. We sampled the descending larvae in a pine stand, 'Oldebroek', in the same area about 40 km from 'Plijmen', and in some years the population densities obviously differed (Table 46).

On what order of magnitude is the advantage gained by density-related moth dispersal? A rough estimate can be obtained from the following data in Klomp's paper (1966, figs. 26 and 36):

mean fecundity, at low density (2 larvae/m²): 200 eggs/female
mean fecundity, at high density (26 larvae/m²): 150 eggs/female
generation survival, at low initial density (5.4 eggs/m²): 2.8%
generation survival, at high initial density (136 eggs/m²): 0.16%

The maximum advantage offered by dispersal, then, can be estimated as follows:

Without a density effect (i.e., no reduction of fecundity and no dispersal), one female would lay 200 eggs at high density, thus giving rise to 0.32 new adults; with a density effect (i.e., reduction of fecundity and dispersal), one female arising from high density and depositing its whole supply of eggs after dispersal into a place of low density, would lay 150 eggs, which would give rise to 4.2 new adults.

Thus, the density effect would be advantageous if, on the average, more than one out of 14 females emerging in a densely populated area were to succeed in depositing their eggs in underpopulated forests.

4.4 Conclusion

We can now return to the surmise put forward in the general introduction, that the effects of mutual interference may serve to regulate population density at a low level. In the present chapter, the hypothesis has been developed that the function of the density effect is to increase moth dispersal. Should it also be considered as a mechanism for self-regulation of population density? To answer this question, two points must be laid down.

The first is that the answer depends on the definition of population. With a limited definition, i.e. when the various parts of a pattern of locally variable densities are considered as separate populations, the density effect must have some regulatory effect, at least theoretically. That its impact is insufficient to explain the observed regulation of local population density (Klomp, 1966) is another story.

If, however, the definition of population density is extended to include those local groups between which a more or less frequent mutual exchange of individuals occurs, the density effect cannot be endowed with a regulatory influence. Reproductive capacity is withdrawn from places with high density for the sake of its fuller realization in other places; hence, the capacity for increase is not really restricted.

In my opinion, it is rather immaterial which definition of population is chosen, if only the way in which a study is conducted permits the detection of the processes going on.

The second point is more relevant. If density were homogeneously high for a certain number of generations and over an extensive area, density-related dispersal would bring no advantage to the individual and the density effect would disappear through selection.

Therefore, I suggest that population regulation is brought about by classical density-related processes (such as predation and parasitism) and that the density effect is an adaptation by which mortality from these processes can be escaped by exploiting the heterogeneity of density occurring over extensive areas.

Summary

In a field population of *Bupalus piniarius* in the Netherlands, Klomp (1958a, 1966) found a negative correlation of population density with growth and adult fecundity. The experimental analysis of this relationship in *B. piniarius* is reported in this paper.

Part I describes growth of isolated larvae, under the influence of abiotic factors. The experiments were performed at the former Institute for Biological Field Research (now amalgamated into the Research Institute for Nature Management) at Arnhem, in an outdoor insectary, the laboratory, and a greenhouse.

The number of larval instars varied from four to eight, according to environmental conditions and sex. Warm conditions and long photoperiods tended to increase the number of instars. Females tended to moult one time more than males. Under given environmental conditions, the variation in number of instars was rarely more than one. In the field, growth proceeds through five or six, and exceptionally through four, instars.

Rate of growth was positively correlated with temperature, especially during the earlier larval stages, and negatively with photoperiod, particularly during the older larval stages. Warmth (e.g., 25° C) and long photoperiods (e.g., 17 hr) became detrimental towards the end of the larval stage.

It is concluded that temperature and photoperiod influence the number of instars in *Bupalus* directly, rather than indirectly via the rate of growth.

The period during which external factors determined the ultimate number of larval instars began in the embryonic stage and ended about the middle of the larval stage. The possible endocrine mechanism determining the number of instars is briefly discussed.

Average pupal weight was positively correlated with number of larval instars. For a given number of larval instars, pupal weight was: 1) positively correlated with average temperature during the larval period, within the range of temperatures that permit normal development; 2) reduced by rearing in constant darkness. Slight differences in daylength during the larval stage were not reflected in pupal size.

Morphologically normal pupae were formed after development through four, five, six, and seven larval instars. Moths of four, five, and six-instar larvae were capable of normal reproductive behaviour; seven-instar larvae were not tested in this respect. Sixinstar females produced more ripe eggs than five-instar females. The number of eggs laid did not differ significantly between the two groups, owing to a greater retention of eggs in the former group. No differences in viability of eggs and newly hatched larvae were found between these two categories.

Part II of the present paper reports an experimental analysis of the negative correlation of population density with growth and fecundity in *Bupalus piniarius*, and an attempt to evaluate its function. The majority of the experiments, like those of Part I were performed at the former Institute for Biological Field Research. Some were conducted in the field.

Both laboratory and field experiments confirmed the causality of the negative correlation between larval density and growth. In both laboratory and field, the maximum effect was attained at quite low densities, and when food was abundant.

Aggregation under experimental conditions, if not excessively high, did not affect either mortality of larvae and pupae or longevity of the adults. In the first larval instar (L1), aggregation did not influence growth. In all other stages except the last, it retarded and reduced growth, and reduced the efficiency of conversion of food absorbed through the intestine. In the last larval stage, the rate of growth and efficiency of food conversion were about the same in aggregated and in isolated larvae, but weight of the former remained lower because aggregation reduced the duration of this stage.

In some identical experiments, the average reduction in pupal weight due to crowding varied from 12 to 24% in females and from 9 to 17% in males.

The females showed significant individual differences in the effect of aggregation on growth, probably reflecting genetic differences in susceptibility to the density effect.

Aggregated larvae tended to develop through one fewer instar than isolated specimens. This is probably an indirect effect of retarded development caused by crowding.

The behaviour of the larvae is described in some detail. They rested for by far the greater part of the day. Activity was restricted mainly to the night, especially during twilight, except in L1, when there was considerable diurnal activity. Crowded L1 larvae seemed somewhat more mobile than isolated specimens, but behavioural differences between the two categories could not be detected in the other larval stages.

A preliminary analysis of the chemical composition of pupae showed no obvious differences between density categories in content of dry matter, carbohydrates, fat, and nitrogen.

Moth eclosion was slightly retarded after larval crowding.

In the adults, all the linear dimensions considered (including wing loading) were reduced by about the same degree by larval crowding.

Fecundity of females was reduced by larval crowding, as expected from its positive correlation with pupal weight. Females from aggregated cultures laid somewhat smaller eggs, and produced more eggs per unit body weight, than those reared in isolation. Egg retention was reduced by grouping of the larvae. On average, however, fewer eggs were actually laid by females from aggregated cultures.

Tests of offspring viability relative to density conditions in the parental generation yielded contradictory results. All the evidence presently available disproves an earlier

conclusion (Klomp & Gruys, 1965) about reduced viability as a result of aggregation.

An analysis of field data showed that larval density had a much greater influence on growth than did temperature and daylength, whose effect in the field proved to be insignificant. (Chapter 2)

The stimulus that the larvae exert upon each other did not consist of contamination of the food nor did it involve olfactory or visual perception. Only when the larvae had direct bodily contacts with each other during the night was growth reduced. Growth was not reduced if such contacts were restricted to the daytime.

Regurgitated fluid gut contents, transferred between one another during contacts, were an essential component in the growth-reducing stimulus. Mere mechanical disturbance (to which the larvae showed the same behavioural reactions as to disturbance by other larvae) had no effect on growth.

It is concluded that the essential stimulus induces a physiological change which reduces the rates of feeding and of growth; direct interference with feeding, as a result of mutual disturbance, is not the primary cause of growth reduction.

Even when grouping was restricted to a relatively short period (i.e. one of the larval stages, or five days out of a total larval life of 100 days) there was a lasting reduction in size except when aggregation was restricted to L1.

The number of encounters required for growth reduction is rather low, and the area of pine foliage visited by half-grown larvae is sufficient to permit the occurrence of effective mutual interference at the intermediate and high densities found in the field.

The induction of the density effect is not completely specific. Of four species of pine-inhabiting larvae examined, one geometrid reduced growth as effectively as *Bupalus* itself. (Chapter 3)

The expression and the underlying mechanism of the density effect in *Bupalus* are compared with similar phenomena in other insect species, and its function is discussed.

High larval density may increase the tendency of female moths to disperse. Density-induced reduction of weight would at least contribute to the realization of this tendency and may even be one of the causes of the increased tendency to disperse. Dispersal from highly populated areas, with a fair chance of reaching areas of low population density, presumably enhances the chance of survival of the offspring. Reduction in fecundity owing to high density seems a small price to pay for more effective reproduction. Some points relevant to this hypothesis are discussed (adult dispersal in relation to larval population density; local variations in population density; survival in relation to population density).

It is tentatively concluded that, rather than being a mechanism for self-regulation of population density, the density effect is an adaptation that avoids mortality due to density-related processes by exploiting the heterogeneity of density occurring over extensive areas. (Chapter 4)

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