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The Ecological Station of Uppsala University on Öland 1963–1973
A brief presentation

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Ecology—scope and aims
Ecology is the study of the relation of organisms to their environment. Thus ecology is concerned with cause and effect in nature. Organisms and their environment function together as a system: an ecological system, or “ecosystem”. The theory of ecosystems has gained progressively wider recognition and, during the past two decades, has gained a place in public awareness in all environmentally conscious societies.

Ecology has acquired vital importance as a field of biological research. Its findings offer guidance to community planners when interference with nature is unavoidable. Broad acceptance is now accorded to the view that man must cease his arbitrary dictatorship over the world’s fauna and flora, on whose existence he himself is totally dependent. Ecology draws on all the other divisions of biology, linking together their findings and thus constituting an "umbrella" discipline. In all research into the future, ecology occupies a place of prime importance.

As a word, ecology is relatively new; but the concept is older. Linnaeus spoke of the “economy of nature” and Darwin’s theory of evolution has contributed importantly to the formulation of ecological principles, and has enriched their content.

The aims of ecological research are to find the answers to three principal questions:
1. how can various lifeforms—species—live together in a confined environment?
2. how have the different forms arisen?
3. how can man utilize nature without damaging her?

Life is characterized by the ability to reproduce; individuals by excitability, i.e. response to stimuli. Interaction in nature—between various living organisms and between these organisms and their environment—depends on the ability to react in a definite way to given stimuli. Reproduction and excitability are the keys to the capacity of a species to occupy an environment in competition.

Ecological research falls under three main headings: biocenology, or the study of relations between living organisms in a community; biogeochemistry, or the study of the distribution and circulation in nature of the chemical elements essential to life; and bioenergetics, which is the study of energy flow in the biosphere, i.e. the layer surrounding the earth in which living organisms are found.

In the light of the two basic characteristics of life—the ability to reproduce and to react to stimuli from the environment—ecology can focus research into living nature on two important aspects: the quantitative aspect of flora and fauna, i.e. population conditions and location on the earth, and the behavioural aspect, meaning the dependence of plants and animals on environmental stimuli for dispersal, location, coexistence and reproduction.

Ecological research commences with the observation of nature, continues with laboratory experiments, and the results of these are then checked and tested in nature. All ecological research starts and finishes literally in the field! Previously, ecological field stations were very simply equipped for observations and the collection of live specimens. During the present century, reliability criteria for scientific facts have been greatly sharpened. Modern ecological stations must be equipped with specialized apparatus and, basically, differ from other biological research establishments only in that they have living accommodation. By these standards, the Ecological Station of the University of Uppsala, on the island of Öland off the East Coast of Sweden, is a good contemporary example.
Work at the station was originally concerned mainly with two projects:
- chemical communication in the insect world and between insects and plants
- the dependence of insects on weather and climate factors.

These central areas of research had already been attacked on the island in the form of pure biological field activity, which is related to the fact that the natural environment and climate on Öland are extremely suitable for such fundamental scientific work.

Environment
Öland is a long, low island—130 km from north to south and 18 km wide at the broadest point. Located in the southern Baltic near to the East Coast of Sweden, Öland enjoys a relatively sunny and dry climate due to the proximity of the South Swedish Uplands. The island climate evidences both continental and maritime features and in the summer the local, or microclimatic, conditions at places may exhibit wide daily variations in temperature and humidity. In such cases, relatively low humidity is usually encountered close to the ground.

During the warmer months of the year, the weather on Öland is relatively favourable for field experiments concerning plant and animal life. The bedrock consists largely of Ordovician limestone in its upper layer and at several places in the southern part of the island there is a top cover of decomposed rock formed in situ. Elsewhere, and particularly in the low-lying strip parallel with the sound on the mainland side, a rich spectrum of glacial and post-glacial soil types is encountered. The limestone plateau constituting the major part of the island has a relatively thin soil covering.

Due to the mild general climate, richly varied microclimate, and the soil types, the vegetation is full of variety and comprises many species, despite the relatively moderate size of the island. This vegetation favours the survival of a varied fauna.

Research programme and equipment
Some years before the advent of the ecological station (1950), the present authors, who are entomologist and biochemist, respectively, entered into direct co-operation on a biological chemical research project concerned with the biological function and chemical structure of natural odorous compounds.

Thus, when the two first-mentioned projects made increasingly urgent demands for a properly equipped station, plans were prepared accordingly. In 1961, the Axel and Margaret Axson Johnson Foundation granted funds for the establishment of a terrestrial-ecological research station on Öland, under the ægis of Uppsala University. Further generous donations from the Foundation have permitted extensions to the station and provided its equipment with specialised apparatus of particularly high standard. Typical of the work of the station right from the start have been a team spirit and co-operation which take no recognition of traditional boundaries between disciplines or institutes.

With the passing of the years the Ecological Station of Uppsala University on Öland has come to house four major research projects:
1. Biological function and chemical structure of natural odorous compounds.
2. Bioclimatology of insects.
3. External parasites of the hedgehog.
4. Terrestrial phyto-ecology with reference to the peculiar vegetation of Öland: the relatively luxuriant deciduous forest types and the alvar vegetation, comprising varied series of heath, dry meadow and steppe-like ecosystems.

It must be added that many scientists working in the wide area of ecology have taken the opportunity of visiting the Ecological Station on Öland for short-term stays in order to collect study material in the field for further work at other institutes.

The following are among the more important instruments and equipment acquired by the Ecological Station on Öland:
- LKB 9000 combination gas chromatograph/mass spectrometer with precolumns; Becker-Ryhage-Stenhagen jet separator.
- Perkin-Elmer 900 gas chromatograph with glass capillary columns and precolumns.
- IR spectro-photometer, type Perkin-Elmer 457: ultra microcells, gas cells, beam condensor.
- Visible light/ultraviolet spectro-photometer, Bausch and Lomb, type Spectronic 505.
- Thin-layer and columnar chromatography equipment.

1 The Ecological Station of Uppsala University on Öland was inaugurated on July 8, 1963 by the Vice-Chancellor of the University of Uppsala in the presence of the County Governor and members of the Board of the Axel and Margaret Axson Johnson Foundation.
Molecular distillation apparatus.
Several types of rotary vacuum pumps.
Equipment for hydrogenation, ozonizing and silylation.
Micromanipulator (Leitz).
Wild binocular photomicroscope.
Wild binocular photomicroscope with IR and UV converters.
Meteorological equipment.

The colour photographs reproduced here will give some information about the Ecological Station on Öland, its disposition, equipment, and main research projects.

We hope that the series of papers presented in this ten-year jubilee volume will show on the one hand the diversity of the scientific work at the Ecological Station on Öland, and on the other, the thread running through all its activities: the common interest in interaction between organisms—species—in the ecosystems.
Plate 1
2. The main library.
3. The greenhouse and out-of-doors plantations of insect-attracting plants.

Plate 2
1. Using the capillary gas chromatograph.
2. Using the combination gas chromatograph–mass spectrometer.
3. Thin-layer chromatography is recorded by the aid of polaroid photography.

Plate 3
1–3. Field experiments with aculeate Hymenoptera males.
1. Artificial flying paths for Gorytes males, made by trampling the herbaceous plants.
2. For the purpose of experimental studies trimmed bushes in a flying territory of bumble-bee males.

3. Dummy tree stump for experiments with Bombus hortorum.

Plate 4
1. Grasshoppers sunbathing in autumn.
2. The body temperature of lygaeid bugs is recorded with the aid of thermocouples inserted anally.
3. Laboratory experiments with the reaction of hydrophilids (Helophorus brevipalpis) on change of temperature.

Plate 5
1. Collecting fleas from a hedgehog.
2. A station for the study of the alvar vegetation.
3. A station for the study of the deciduous woodlands.

Plate 6
1. Filming the scenting behaviour of bumble-bees (Psithyrus rupestris and Ps. bohemicus). Öland.
2. Electronic flash photographing of experiments with Eucera tuberculata. Languedoc.
3. Filming experiments with E. grisea. Majorca.
New Observations on the Pollination of Ophrys L. (Orchidaceae)

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ABSTRACT

Kullenberg, B. (Entomological Department of the Zoological Institute and Ecological Station of Uppsala University on Öland, Uppsala and Färjestaden respectively, Sweden.) New observations on the pollination of Ophrys L. (Orchidaceae).


In addition to the results of observations and field experiments in Sweden, Morocco, Algeria and Lebanon, published in 1961, new observations from Southern France, Majorca and Southern Sweden are reported. In addition to the same species as before, O. bertolonii has also been studied. It has been established that even in these regions the pollination is principally effected by the same types of aculeate Hymenoptera males, sexually excited by the scents of the flowers. Occasional visits, probably restricted to certain localities, to the flowers of certain species by scarabaeid Coleoptera have been observed and the role of this pollination is discussed.

INTRODUCTION

In some earlier works, the author (Kullenberg, 1952, 1956, 1961) stated that the effective pollinators of a series of Ophrys species (family Orchidaceae) are males of certain aculeate Hymenoptera, which are attracted by the flower scents. At the same time, this chemical stimulation physiologically predisposes the males to a mood of copulatory activity. In other words: these Hymenoptera males are apparently influenced by the Ophrys flower scents as if these were sexual pheromones. Ophrys flowers do not secrete nectar, thus foraging insects with principally licking-sucking mouth-parts do not regularly visit the flowers and cannot be bound to them with regard to food.

The male insect, settled on the flower labellum, performs movements that are tactilely stimulated and guided by the superficial structure of the upper surface of the labellum. This behaviour is a simulat of the behaviour of attempted copulation. It must be stressed that this "false" attempted copulation apparently cannot be initiated without the olfactory stimulation from the flower scent.

As regards the final position of the male insect on the Ophrys labellum, there are two types. Most Ophrys labella guide the male Hymenopteron so as to settle with the head upwards, the pollinia thus being taken with the head. Unlike all other species, O. lutea and O. fusca constrain Andrena males to turn after alighting and take the pollinia with the tip of the abdomen.

The observations on the floral ecology of Ophrys reported in the monograph of 1961 were made in Sweden, Morocco, Algeria and Lebanon. This paper deals with field observations made in Majorca, Languedoc (Southern France) and Öland (Southern Sweden).

OBSERVATIONS

O. Insectifera L.

As effective pollinators, I have up to now observed only two sphecid wasps, Gorytes mystaceus L. and G. campestris Müll. (=fargei Shuckard). The last-mentioned has two colour varieties on Öland, one with yellow markings and another with cream-white markings (Plate 1, 1–3).

It has already been stated (1961) that copulatory behaviour on the labella is influenced by the form of the latter. For example, a labellum somewhat like that of O. speculum regis ferdinandii Achtaroff et Kellerer seems to bring about quieter behaviour than does a broad labellum. I once observed an unusual case—a broad labellum with a central lobe having a distal border with an undulating edge, provoking eager groping movements of fairly long duration with the tip of the abdomen.
O. speculum Link

The species is very common on Majorca, occurring abundantly in several biotopes that are not too shady. It is probably the commonest Ophrys species on the island. The only pollinator observed on Majorca, as in Algeria and Morocco, is the scelid wasp Campsoscolia c. ciliata F. This species seems to be the most common aculeate Hymenopteron on the island of Majorca in the month of March (Plate I, 4).

The pollination behaviour here is the same as that reported by Kullenberg in 1961. Nothing seems to be known about the host of the larvae of Campsoscolia c. ciliata, but as the latter can sometimes be seen to hatch in rather large numbers from one spot it might be supposed that big scarabaeid larvae serve as host insects. Two big species, Scarabaeus laticollis L. and S. semipunctatus F. are rather common on Majorca, and S. sacer L. may also be met with.

In spite of very strong dependence for its pollination on the male Campsoscolia c. ciliata, O. speculum can sometimes be observed to hybridize. This depends on the obvious capacity of Campsoscolia c. ciliata to be olfactorily stimulated even by some other Ophrys species though never in the strongly exciting way characteristic by O. speculum. I once found a variety of O. speculum with a whitish mirror patch and yellowish-brown border hairs. These flowers had a distinctly weaker scent-stimulating ability than normal flowers. Given below is a list in rank of Ophrys forms with respect to their ability to attract the male Campsoscolia c. ciliata by scent. The list was compiled after experimental observations made on Majorca.

1. O. speculum (normal form)
2. O. speculum × O. sphecodes atrata, O. bertolonii
3. O. speculum, form with white mirror patch and yellowish-brown border hairs
4. O. speculum × O. bombyliflora
5. O. fusca (normal form), O. sphecodes atrata

Flowers of O. apifera did not seem to provoke approaches.

O. fusca Link

On Majorca I observed the Andrena flavipes Pz. male to be a pollinator of this Ophrys species. I did not see it on the flower of O. fusca versus omegaflora Fleischm. which flowers later than the other O. fusca forms on Majorca. Mr G. July, (Wörth, Donau, West Germany), kindly informed me by letter that he saw A. flavipes as a pollinator of O. fusca in Cyprus. This Andrena species is widely distributed in Southern Europe.

O. lutea Cav.

In Languedoc I saw as pollinator the male of Andrena cinerea Brullé (determination by K. Warncke). Observations during experimental conditions on Öland revealed that Andrena nigroaenea K. males are olfactorily and tactiley stimulated to copulatory attempts by the flower labellum. Anyhow, it seems as if O. sphecodes atrata flowers chemically stimulate this Andrena more than do O. lutea. A. sericea Christ males are normally stimulated to copulatory attempts by the labellum, like the male of A. lathyrifl. A., but A. haemorrhhoa F. may be regarded as subnormally stimulated.

O. sphecodes Mill. s. lato

The taxonomic problems regarding different forms of this polytypic taxon will not be dealt with in this paper. Experimental research on the floral ecology of different forms of the sphecodes-group as well as chemical research on the flower scents are in progress.

O. sphecodes litigiosa (E. G. Cam.) Becherer

Mr J. Forster, Winterthur, Switzerland, kindly informed me by letter about his interesting observations on the pollination of O. sphecodes litigiosa by the male of an elaterid beetle, apparently sexually stimulated by the labellum of the flower. Andrena nigroaenea and A. haemorrhhoa males are attracted by the scent of the flowers, though the tactile stimulation of the labellum must be regarded as being probably subnormal.

O. sphecodes atrata (Lindl.) E. Mayer

Observations during experimental conditions show that Andrena nigroaenea males are strongly attracted to the flowers by the scent and tactiley stimulated by the upper surface of the labellum to copulatory attempts.

O. sphecodes provincialis Nelson

This form flowers early in Languedoc and during the same period as O. arachnitiformis and O. sphecodes Mill. In one of my study localities all
three types of Ophrys are found to grow abundantly together. I once observed the male Colletes cunicularius L. (Apidae) to be chemically and tactically stimulated by the flower of O. sph.provincialis and to be effective as a pollinator (Plate 1, 5).

O. arachnitiformis Gren. et Phil. is known to be visited by the male Colletes cunicularius and field experiments with labellum extracts of the latter three Ophrys forms show that the male Colletes cunicularius is strongly attracted by their scent in the way characteristic of the chemical attraction of aculeate Hymenoptera males by Ophrys scents. Evidence of the same chemical and behavioural constitution in certain Andrena species has been obtained. I do not find it surprising that morphologically these Ophrys forms are difficult to distinguish from each other.

O. bertoloniir Mor.
This species is rather common on Majorca. In some localities the morphology of the flower seems to indicate genetic influence from O. tentredinifera. I have seen one hybrid population with good representatives of the hybrid between these two species, the so-called O. ×kallista G. Keller. Insect visits on the flowers of O. bertoloniir are not often seen. Nevertheless on Majorca I have had the opportunity of observing visits to the flowers by the males of Campsoscolia c. ciliata and Eucera nigrilabris Lep. (Plate 2, 1–2). The males seemed not to be quite normally stimulated to copulatory attempts with the labellum, though the chemically excited approach flight was performed as for the Ophrys species they normally pollinate. The male E. grisea F. is but weakly attracted to O. bertoloniir. In the same locality I also saw the lamellicorn beetle Oxthyrnea funesta Podá visiting the flowers on several occasions. This Coleopteron may more or less randomly bring about pollination. The observations briefly reported here on insect visits to the flower of O. bertoloniir are of interest from the point of view of the known hybrids between it and O. speculum and O. tentredinifera respectively.

O. apifera Huds.
Field experiments in Languedoc show that the male Eucera tuberculata F. is attracted to this Ophrys species in the same way as to O. scolopax. (See also the paper on electrophysiological experiments written by Priesner, 1973.)

O. tentredinifera Wild.
On Majorca the male E. nigrilabris is a frequently appearing pollinator of this species (Plate 1, 7; Plate 2, 8). E. grisea males may perform loosening of pollinia and pollination, but it seems rather a rare phenomenon as these Eucera males are apparently less attracted by the scent of O. tentredinifera than E. nigrilabris. Furthermore, the body size of E. grisea is generally too small in relation to the size of the flower labellum to make possible contact between the bases of the pollinia and the head of the insect during the attempted copulation.

The male E. numida Lep., rarely observed among flying males of E. nigrilabris does not seem to be attracted to the flower of O. tentredinifera.

O. fuciflora (Crantz) Moench
Mr J. Forster, Winterthur, Switzerland, told me in a letter about interesting observations on the pollination of this species by two lamellicorn Coleoptera, Hoplia larinosa L. and Phylloptera horticola L. The beetles did not seem to be sexually excited. He also observed the visit of the male E. longicornis L. to the flower of O. fuciflora (cf. Kullenberg, 1961).

O. scolopax Cav.
In Languedoc I have seen this species being pollinated by the male E. tuberculata. E. caspica Mor. does not seem to be ecologically related to O. scolopax. Cf. Plate 1, 6, a male E. longicornis on a flower of O. scolopax.

O. bombyliiflora Link
This species is perhaps as numerous and as widely distributed on Majorca as is O. speculum. Its only observed pollinator is the male Eucera grisea and this Eucera species seems to be almost as frequent as Campsoscolia c. ciliata in the months of March and April (Plate 1, 8). The E. nigrilabris male often seen together with E. grisea is apparently to a lesser degree attracted to the flower of O. bombyliiflora than the E. grisea male is to the flower of O. tentredinifera.

Regarding the attractiveness of certain Ophrys flowers for E. grisea males I refer here to a rank list given below, based on observations in March–April 1971 on Majorca.
1. O. bombyliiflora
2. O. tentredinifera ×?, alt. O. tentredinifera var. (perhaps influence from O. bombyliiflora)
3. *O. tentredinifera, O. apifera*
4. *O. bertolonii* versus *O. × kallista* (perhaps introgression *O. tentredinifera × O. bertolonii*)
5. *O. bertolonii*

A supposed hybrid form *O. speculum × O. atrata* did not seem to provoke approaches.

*O. reinholdii* Fleischm.

Mr G. July, Wörth/Donau, West-Germany kindly told me in a letter with colour photographs about his very interesting observations on the pollination of this species by the male of a *Meloea* Latr. species. The pollinia are taken by the head of the bee. As far as I know it is the first observation of insect-pollination of an *Ophrys* species belonging to sectio Orientales Nelson.

**DISCUSSION**

The briefly reported new observations on the pollination of the *Ophrys* orchids confirm the results of earlier studies by the present author, collocated in the monograph of 1961. Thus regular pollination on Majorca and in Languedoc is carried out as in other parts of the distribution area of the genus by males of certain species of Hymenoptera Aculeata—of the same genera. The insects alight on the flowers, sexually excited by the perfumes of the labella (cf. Plate 2, 8 and 9), and guided by the superficial labellum structures, perform movements of simulated copulation. G. July has made the new and interesting observation that an *Ophrys* species is pollinated by the male of a species of *Meloea*. Hitherto, no species of nest-parasiting Hymenoptera have been known as *Ophrys* pollinators. The behaviour of the males brings about pollination if the size ratio flower—insect does not interfere. The existence of what the present author calls a "concealed" or "secret" pollination by lamellicorn Coleoptera is also confirmed. Mr J. Forster reports something really new—the pollination of *O. sphecodes litigiosa* by the sexually excited male of an elaterid Coleopteron. The following types of males *aculeata Hymenoptera* have thus hitherto been observed as *Ophrys* pollinating: *Campsonotus* (1 species), *Gorytes* (2 species), *Colletes* (1 species) *Andrena* s. lato (several species), *Eucaera* and *Tetralonia* (several species), *Meloea* (1 species). The appearance of females of these Hymenoptera types is shown in Plate 2, 3–7 and 10. The lamellicorn Coleoptera, such as *Oxythyrea funesta* frequent the reddish flowers of *Cistus albidus* L. for foraging in March and April on Majorca. As certain *Ophrys* species, such as *O. tentredinifera* and *O. bertolonii*, have reddish sepals, it is possible that beetles, bound for food to the flowers of *Cistus*, react positively to the coloration of the sepals of these *Ophrys* species. Having alighted on the flowers they may, perhaps somewhat clumsily, perform pollination more or less at random. These scarabaeid beetles are rather numerous in certain localities.

In such biocoenoses the *Cistus* species might be regarded as secondary ecological support organisms to the *Ophrys* spp. in question, because of their ability to hold the lamellicorn beetles available as auxiliary pollinators—as "pollinators in reserve".

**ACKNOWLEDGEMENTS**

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**BIBLIOGRAPHY**


— 1961. Studies in *Ophrys* pollination. Ibid. 34.


Vecht, J. van der, 1928. Fauna van Nederland, aflevering 4, Hymenoptera Anthophila (Q XIII m) A. Andrena. Leiden.
Plate 1

3. Male G. campestris (white-banded form) pollinating flower of O. insectifera.
5. Male Colletes cunicularius on flowers of O. sphecodes provincialis.

Plate 2

1. Male Campsoscelia ciliata in copulatory attempt with a labellum of Ophrys bertolonii.
2. Male Eucera nigrilabris approaching a flower of O. bertolonii. The male Eucera is carrying pollinia of O. tenthredinifera.
3. Female Gorytes mystaceus.
4. Female Andrena haemorrhhoa.
5. Female Campsoscelia ciliata.
6. Female Melecia lucresia.
7. Female A. ovata.
8. Males of E. nigrilabris are strongly attracted by flowers of O. tenthredinifera.
9. Males of Colletes cunicularius gathering at a point where a female is hatching.
10. C. cunicularius pair in copulation.
Territorial Flight of Bumble-bee Males in Coniferous Forest on the Northernmost Part of the island of Öland

BERIT BRINGER

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ABSTRACT

Bringer, B. (Entomological Department of the Zoological Institute and Ecological Station of Uppsala University on Öland, Uppsala and Färjestaden respectively, Sweden.) *Territorial flight of bumble-bee males in coniferous forest on the northernmost part of the island of Öland.* ZOON, Suppl. 1: 15–22, 1973

The territorial flight of *Bombus* (Latr.) and *Psithyrus* (Lep.) males has been investigated in coniferous forest on the northernmost part of the island of Öland. In this forest, flight territories with a great number of males (*Bombus* and *Psithyrus* spp.) alternated with wide areas of similar appearance where no males at all were found. The observations of flight behaviour have resulted in diagrams showing flight levels of *Bombus* and *Psithyrus* males and maps showing the flight paths of colour-marked males of different species.

INTRODUCTION

The habit of bumble-bee males of flying along fixed paths and marking their flight paths with scenting materials, has caught the interest of several scientists, since Darwin in September 1834 discovered territorial flight of the male *Bombus hortorum* L. in the garden of his son.

My own investigations started in 1964. The observations have been made principally in an area situated in the parish of Böda on the northernmost part of Öland. The research area is to a large extent covered with coniferous forest (pine and spruce), more or less mixed with deciduous trees and often rich in herbs.

Several entomologists (Bachmann, 1915; Frank, 1941; Haas, 1949a) have stated that the young males leave their nests forever on reaching adulthood. In my area they are found asleep in flowers in the afternoon and in the night from the latter part of July. The meeting with the young queens should therefore take place somewhere outside the nests. In earlier descriptions of territorial flight (see Frank, Haas, and Krüger) it is mentioned that the males, after leaving their nests, assemble in limited areas. In the forest of Böda, flight territories with a great number of males alternated with wide areas of similar appearance where no males at all were to be seen. In every flight territory observed, males of several species of *Bombus* and *Psithyrus* could be seen flying together. In one locality flight paths of all species of the area investigated, except *B. lucorum* L. (dark form), were observed.

FLIGHT INTENSITY

The flight of all bumble-bee males is more or less dependent on the weather. On calm, warm, sunny days the flight began earlier and continued longer, while on clouded, cool and windy mornings the start was delayed or did not occur at all. The males of all species studied displayed maximal activity in the morning. For most of the species territorial flight appeared between 8.00 and 12.00 with minor divergences both early and late. Contrary to the *Bombus* species, the *Psithyrus* males were very dependent on direct sunshine to be able to begin the day's flight. The *Bombus* males normally also started a little earlier in the morning and continued a little later in the day than *Psithyrus*. On cool, cloudy days, males of e.g. *B. hortorum* and *B. hypnorum* L. showed weak activity for one or two hours while the *Psithyrus* males did not even leave the flowers. *B. hortorum* and *B. pratorum* L. seemed to withstand bad weather better than other bumble-bees and were sporadically observed.

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1 Two forms exist, “dark” and “blond” (cf. Bergström et al., 1973).
in territorial flight in mist and drizzle. When on sunny days, with much flight activity by both Bombus and Psithyrus, a cloud veiled the sun, the Bombus males continued their flight while the Psithyrus males immediately settled on the vegetation or the ground until the cloud disappeared. On very warm, sunny days the Bombus species mentioned sometimes displayed a feeble flight activity until 4 p.m. On sunny mornings flight intensity increased from the start to a maximum at about 10 a.m.

**FLIGHT LEVEL AND FLIGHT BEHAVIOUR**

Hybridizing of allied species may be prevented in several ways. In certain cases such allied species live in separate habitats or their activity is concentrated to different times of the day. Should they not be separated either in time or in space, the structures or behaviours concerned with signal function have very often been favoured during the evolution of the species in a specific manner which separates the allied species.

As the activity of males of all species was at its climax in the morning, the diurnal time of activity cannot be an isolating factor. I have also mentioned that different species are normally found flying together in the same biotope. The question is: how is species isolation maintained in such a biocoenosis?

**The Genus Bombus**

Haas (1949a) tried to classify the species studied by him according to their choice of flight level. Diagrams 1 and 2 show the flight level of the Bombus and Psithyrus species according to my observations. My diagram for Bombus agrees very well with that of Haas (1949a, p. 303). Both show that different species can partly use the same flight level. To illustrate how the choice of habitat nevertheless can have a species-isolating function I will give some examples from my own observations.

*B. pratorum* and *B. lucorum* "blond" existed side by side in most localities within my investigation area and normally used a flight level between 2 and 4 metres. In the choice of trees for their approach points they showed clear differences. *B. lucorum* often approached the spruces, though pines and deciduous trees were also included in their flight paths. None of the species investigated was as strictly associated with a special kind of tree as *B. pratorum* is to spruce, when this tree was present. In the choice of tree, *B. lucorum* "blond" preferred thin narrow spruces 6–9 metres in height, where the bumble-bee in a characteristic way slowly ascended vertically close to the trunk from 2–3 m up to 5–7 m. Then the male continued in a swift horizontal or sinking flight to the next tree where he again rose alongside the trunk. In a sparse type of forest, e.g. a young pine wood, the paths were much lower than in a dense one. There the Bombus lucorum males approached the trunk at a level of 0.25–1.5 metres, ascended to 3 metres and left for the next
tree in a sinking trajectory. *B. lucorum* "blond" could also choose quite tall, wide spruces for their approach. In such trees the trunks had large hanging branches in the level of the flight path. There the bumble-bee followed one of the large branches towards the trunk but would also follow the trunk shorter stretches up to the next branch layer. *B. lucorum* "dark" showed quite another behaviour. The males were observed flying in a young pine forest about 3–4 m above the ground. The trees were approached in a kind of upward spiral flight along the branches in the different branch layers or the males only circled around the branch tips.

*B. pratorum* preferred small and bushy spruces, often in dense parts of the forest where neither of the two types of *B. lucorum* were found. The most common place for their approach points was below the tips of hanging spruce branches. They never approached close to the trunk as did *B. lucorum* "blond".

*B. silvarum* L. and *B. agrorum* Fabr. also partly have the same flight level as the species mentioned. In all localities where *B. silvarum* was observed they avoided denser and darker parts of the vegetation. The males preferred the border zones of the woods where the biotope had a more open and bushy character. The males flew with great speed to a tree where they approached the underneath of a branch tip about 1.5 m above the ground. Then they continued upwards through the branches and leaves, as a rule not close to the trunk like *B. lucorum* "blond", but with a few new approaches through the foliage. In this way they made a swift spiral upwards through the bush or tree. A few times I saw a male swiftly following the trunk upwards but this behaviour was not typical of *B. silvarum*. On the level where the bumble-bee left the tree or the bush, the flight path continued to the next approach point in an arc down towards the level of 1.5 m above the ground. The flight path described by Haas (1949a, p. 289) also passed through an open part of the forest.

I have only sporadical observations of the flight paths of *B. agrorum* and thus find it difficult to compare the choice of habitat of the *B. agrorum* males with other species flying at the same level.

According to the investigations of Haas (1949a) and Krüger (1951) and to my own observations it is obvious that the males of *B. lapidarius* L. seek the highest part of the vegetation for their territory flight. Thus their flight paths were found at the tree-top level. They entered the trees a few metres below the tops and approached the utmost shoots. The males then ascended to the shoot tips of the next branch layers. I could never see any flight within the crown or along the trunk. Finally circular movements were made around the highest branch tips, which was a very characteristic habit of the species.

The two species *B. hortorum* and *B. hypnorum* used the flight level 0–1.5 m above the ground. *B. hortorum* preferred rather dark parts of the forest, rich in tussocks and stubs where the vegetation, because of the sparse light, was low and scant. Their flight paths went close to the ground and seldom ascended above 1 m. The most common location for the approaches were the bases of trunks especially in the angle formed with the roots. The great predilection of *B. hortorum* for such root niches has often been witnessed since the first observations of Darwin 1854. The behaviour of slow, slow flight and the occurrence of the approach points at the bases of the trees also agrees with the descriptions by Frank (1941) and Haas (1949a). Other approach points were the bases of stones and tussocks. The approach points of *B. hortorum* were always situated in the angle between a horizontal and a vertical area, e.g. trunk and ground. The males of *B. hypnorum* also flew close to the ground and preferred root niches as approach points. Both species usually stopped their flight for a short time in the root niche itself, then continued to the next. Sometimes the males approached the bases of the bushes and followed the trunks 2–3 dm upwards. Thus the flight characteristics of *B. hortorum* and *B. hypnorum* are very similar. They often approached the bases of the same trees and bushes and sometimes they seemed to approach the same points. Haas (1949a) also noticed the great similarities between the flight manner of *B. hortorum* and *B. hypnorum* but mentioned that males of *B. hypnorum* only approached the bases of bushes and not the trees and never ascended along the trunks as did *B. hortorum*. These differences do not seem to be valid on Öland.

The behaviour of the males of *Bombus* shows many differences, but there are also some details which are found in more than one species, e.g. the choice of flight level, the spiral flight through the vegetation, and the manner of flying upwards close to the trunks of trees and bushes. It may
be noticed that species with very similar behaviour such as *B. hortorum* and *B. hypnorum* look rather different. (About the differences of the odorous compounds, see Kullenberg et al. 1970.) There seem to be many differences in the choice of habitat and behaviour in territorial flight among the *Bombus* species, which may be factors of importance for the maintenance of the isolation between species.

*The genus *Psithyrus*

The choice of habitat of the *Psithyrus* males is much more uniform than among the *Bombus* species. In many localities I observed how the flight paths of two or more males of the species *Ps. campestris* Panz., *Ps. rupestris* Fabr., *Ps. silvestris* Lep. and *Ps. bohemicus* Seidl passed over the same stretches of ground and created a network of flight paths at the same level in a common flight territory. It appears from Diagram 2 that the *Psithyrus* species (except *Ps. globosus* Eversmann) in my investigation area prefer the level close to the ground for their flight paths. To a great extent the males had their flight territories at the borders of forests and at the roadsides, in meadows and open dwarf-scrub heaths and in areas with occasional large trees, where sunlit and shady areas alternated. In dense vegetation of herbs, ferns and small bushes, the flight paths of the *Psithyrus* species followed the upper surfaces of the foliage, and their flight in such circumstances could reach a level of a few metres.

The *Psithyrus* species seek more open ground for their flight paths which may be due to their greater need of sun for purposes of territorial flight. In very warm and sunny weather the *Psithyrus* males obviously placed their pathways higher.

There is an obvious difference in the manner of approach of the males of *Bombus* and *Psithyrus*. The *Bombus* males approach to a distinct point on the ground or in the vegetation, while the *Psithyrus* species approach a larger area (cf. Sladen, 1912; Haas, 1949b). These approach areas can be situated in the foliage of trees, bushes or herbs, on moss or dwarf-scrub carpets, or on open ground. The approach areas are as a rule horizontal, but on large and dense sunlit spruces, for example, *Psithyrus* males sometimes established vertical approach areas. Over the approach areas the bumble-bees scoured in a slow zig-zag flight (see Maps 7–14). On sunlit ground the distance between the approach areas was, as a rule, rather short, and the bumble-bees flew close to the ground. When the distance was greater the males could rise to 3–4 m and make an arc-shaped pathway between the approach areas. On varying sunlit and shady ground the approach areas were always at a sunlit spot. The size of the areas varied between a few dm² and several m².

I have not been able to follow the flight paths of *Ps. globosus* in detail. From their feeding place the males ascended to the crowns of high spruces. Their flight paths must have been situated far above the ground like the paths of *B. lapidarius* in contrast to all the other *Psithyrus* species studied. If *Ps. globosus* is excluded it seems that the choice of habitat and the behaviour of the genus *Psithyrus* do not show the same variations as those of *Bombus*. It seems difficult or impossible to find any clear differences between the flight behaviour of the *Psithyrus* species *bohemicus*, *silvestris*, *campestris* and *rupestris* which could be supposed to have species-isolating effect.

**FLIGHT PATHS**

To chart the pathways of the bumble-bees, the males were marked with luminous guache colours on the dorsal side of the thorax. The males are in the maps named after these colour marks and the actual year, e.g. orange 66 and yellow 67. Maps 1–4, 6–8 and 11 are from the same locality and 9–13 are from another locality.

<table>
<thead>
<tr>
<th>Map no.</th>
<th>Male</th>
<th>Date</th>
<th>Length of flight path (metres)</th>
<th>No. of approach points</th>
<th>Distance between 2 points, metres</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Yellow</td>
<td>66</td>
<td>195</td>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>White</td>
<td>66</td>
<td>195</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>Orange</td>
<td>66</td>
<td>172</td>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>Orange</td>
<td>66</td>
<td>172</td>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>Orange</td>
<td>67</td>
<td>172</td>
<td>12</td>
<td>1.5</td>
</tr>
<tr>
<td>3</td>
<td>Orange</td>
<td>67</td>
<td>172</td>
<td>17</td>
<td>1.5</td>
</tr>
<tr>
<td>4</td>
<td>Orange</td>
<td>67</td>
<td>172</td>
<td>17</td>
<td>1.5</td>
</tr>
<tr>
<td>4</td>
<td>Orange</td>
<td>67</td>
<td>172</td>
<td>17</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Some males showed a greater consistency in their pathways than others. Orange 66 (Map 2) maintained the leading features of his pathway for a
whole week while orange 67 shifted his pathway every day and also could alter it during the same morning (Maps 3 and 4). Although two points, A and B, are constant on all the days of observation, orange 67 showed a great variability in choice of object for approach. On July 15th and 16th it was mainly the bases of large trees while at the end of the month it was the bases of bushes, tussocks, stones and junipers. All Bombus individuals studied kept the first established flight direction the whole season.

**Bombus silvarum**

As Map 5 shows, this bumble-bee shifted its pathway after 10 o'clock. The morning was very windy on the actual day of observation and the male moved to more protected areas. The length of the pathway was about 100 m and the distance between the approach points varied between 2 and 11 m.

**Bombus pratorum**

As mentioned, this species has its approach points mainly among spruces. The individual of Map 6 also approached fern leaves. The distance between the approach points varied between 1.5 and 29 m. The length of the flight path was 180 m and the number of approach points 21. With very small exceptions the pathway was unchanged from Aug. 2–15, 1967.

**Psithyrus rupestris**

Maps 7 and 8 show the pathways of the male orange 66 on two successive days. Early in the morning orange 66 shared a central approach area (B) with two other males of *Ps. rupestris*, several males of *Ps. campestris* and temporarily with a few males of *Ps. silvestris*. All approach areas were situated in sunlit spots on the ground. As the position of the sun and the bright spots changed, orange 66 also moved his approach areas to new sunlit parts of the ground but the flight direction was constant. The pathway varied a little from Aug. 16 to Aug. 17. On Aug. 16 orange 66 often approached the areas in the order D–A–B–C–D, sometimes also D–A–B–C–A–D. At another locality, a male called white 67 was studied (Map 9). This male had a rather complicated pathway. The approach areas are called A, B, C, D, ... A lap could, in the early forenoon have the appearance B–A–B–E–F–E–G–H–B–C–B. On some laps the male flew from C to D before it returned to B. Sometimes also the loop to G was omitted. After one or two hours the pathway was stabilized and the lap regularly had the appearance B–A–B–E–F–E–G–H–B. Excluding the zig-zag flight over the approaching areas, this pathway had a length of 160 m.

**Psithyrus campestris**

The *Ps. campestris* males white 66 (Map 10), white 67 (Map 12) and red 67 (Map 13) had their pathways along roadsides. At the locality where white 66 had his pathway, the flight of the *Ps. campestris* males was concentrated to two centres at both ends of the territory (station 1 with open grass and station 3 overgrown with ferns). The distance between them was about 175 m. On windy days the male moved his flight path inside the forest curtain where it was impossible to follow. An alderbush (station 2) between stations 1 and 3 seemed to be used not only as a turning point in the pathways but also as a place for sunbathing by the males. Here, white 66 turned back to station 1. Even other males coming from station 3 had their turning points in the alderbush. Other males also flew all the way between station 1 and station 3 and used the alderbush as an approach area on the way. These males must have had a pathway of about 350–400 m.

The pathway of the male red 66 agreed to a great extent with the pathway of the *Ps. rupestris* male orange 66 (Maps 7, 8 and 11). Also the approach areas were partly the same, probably because of the dependence on sunlit spots for the choice of approach areas for both species.

**Psithyrus silvestris**

The pathway of the *Ps. silvestris* male on Map 14 was 280 m. This male had to fly 1 metre above ground for a long way in shadow between the sunlit approach areas. Haas (1949b) mentions that the *Psithyrus* males, unlike the *Bombus* males, never left ground level in straight flight. This does not always agree with my observations. With minor changes this pathway was constant a week later.

**ACKNOWLEDGEMENTS**

The author is greatly indebted to Professor B. Kullenberg, Director of the Entomological Department at the University of Uppsala, for his valuable direction of my work. Thanks are also due to Dr Lennart Cederholm, Lund, for skilful technical assistance, and to my husband Dr K.-G. Bringer for valuable assistance in the field work. Furthermore, the author extends her warmest gratitude to Mr Ted Runeyon for the linguistic revision of the manuscript.
REFERENCES


Observations on Scent Marking by Bombus Latr. and Psithyrus Lep. Males (Hym., Apidae) and Localization of Site of Production of the Secretion

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ABSTRACT

Kullenberg, B., Bergström, G., Brinser, B., Carlberg, B. and Cederberg, B. (Entomological Department of the Zoological Institute and Ecological Station of Uppsala University on Öland, Uppsala and Färjestaden respectively, Sweden.) Observations on scent marking by Bombus Latr. and Psithyrus Lep. males (Hym., Apidae) and localization of site of production of the secretion. ZOON, Suppl. 1: 23–30, 1973

The various procedures of bumble-bee males in applying scent materials in their habitats have been studied in the field in southern Sweden. The observations have been recorded as far as possible by means of 16 mm filming and electronic flash photography. The mandibles have not been observed to play an active part in the application of the scent materials. The underside of the body and the proboscis are active in transferring the marking scents. Refined dissection and analysis techniques make it possible to state that the characteristic compounds of the marking scents are produced in the cephalic part of the labial gland.

INTRODUCTORY NOTES

Since Darwin’s first observations on the regular territorial flying male bumble-bees, many other scientists turned to a study of the phenomenon: Sladen (1912), Frank (1941), Haas (1946, 1949a and b, 1952), Krüger (1951), Kullenberg (1956) and Stein (1956). Sladen stated that bumble-bee males deliver scent products in their flight paths. He assumed that the scent products were species-specific and that the bumble-bee males attract each other as well as young females. (See Plate 1, 7–9, Plate 2.) Haas (1952) was the first to advance an opinion about the techniques of the males when applying their scents to objects in the flight territories. He supposed that the scent material was produced in the mandibular glands. According to Haas, a typical scent-marking object is the edge of a leaf. He says that the male, when applying the scent material, takes the edge between the mandibles, creeps forward along it, and makes biting movements as it proceeds. In Haas’ opinion the movements of the mandibles serve to open the closing mechanism of the ducts of the mandibular glands.

OWN OBSERVATIONS ON PERFUMING BEHAVIOUR

When observing the perfuming activity of male bumble-bees and other aculeate Hymenoptera in the field, we have used the following methods: the naked eye, so-called Gullstrand spectacles, the binocular telescope magnifier with device for close-up observation, electronic flash photography and 16 mm filming. Because of the rapidity of the movements of the perfuming bee, and because of the likelihood of its being obscured behind a leaf, etc., it is difficult to observe details of its behaviour with the naked eye or by conventional optical means. In warm weather the bumble-bees are very swift and thus difficult to observe. The best opportunity for observing the details of the scent-marking behaviour is during sunny weather with relatively cool air. After a variety of observations we have come to the conclusion: no biting movements occur during perfuming activity (Figs. 1–6). The mandibles may be seen to move irregularly—seemingly without any direct correlation with perfuming activity—but also in connection with the activity of cleaning,
Figs. 1 and 3. Male _Psithyrus rupestris_ scenting a grass-stalk. Notice the unclasped proboscis in Fig. 1.

Fig. 2. Male _Ps. rupestris_ scenting fruits of _Filipendula ulmaria_.

Figs. 4 and 5. Male _Bombus lapidarius_ scenting twig and leaf of _Potentilla fruticosa_.

Fig. 6. Male _Ps. rupestris_ scenting the edge of a _Corylus avellana_ leaf.
which frequently accompanies perfuming. In this activity of cleaning the bumble-bee males can sometimes be seen to keep themselves in place by biting the edge or the surface of a leaf (Plate 1, 10). There may be possible variations in the conceivable functions of the mandibles during perfuming—depending not only on individual differences or species, but also on weather conditions, the nature of the substratum to be perfumed or other environmental factors. Besides, movements of the mandibles during perfuming activity as an expression of so-called displacement activity should not be overlooked. We are of the opinion that there are two types of male perfuming activity: active and passive perfuming. Before describing these two types it is necessary briefly to say something about the origin of the marker substances. It seems quite clear that the mandibular glands as well as the cephalic parts of the labial glands produce volatile compounds. We have established that the marker perfume delivered in connection with territorial flying emanates from the cephalic lobes of the labial glands. When perfuming actively the male moves straight forward, in curves or with turns to either side, the under-side of the body against the substratum, and it has sometimes been possible to see that the proboscis is stretched backwards under the body, touching the substratum (Plate 1, 3 and Fig. 1). The labial glands have the orifice anteriorly on the base of the ligula (cf. Heselhaus, 1922; Weber, 1933; Bernard, 1951). In the position observed the proboscis can naturally serve as a brush which coats the odoriferous material. All species observed perfume the leaves and needles of coniferous trees. Perfuming of fine branches and twigs is often seen. Most species observed may perfume low herbs, fresh or withered grass, and different objects in litter. The choice of objects to be perfumed near the ground probably depends partly on weather conditions. *Ps. rupestris* F. males have frequently been observed to perfume trampled—more or less withered—grass during sunny weather with cool air. Some species, such as *B. hortorum* L., *B. hypnorum* L., *B. lucorum* L. and at least sometimes *Ps. bohemicus* Seidl and *Ps. rupestris* are seen to perfume tree stems: the bark with its mosses and lichen or the naked wood of damaged spots (*B. hortorum*). The two first-mentioned *Bombus* species are seen rather frequently to perfume stem-bases, *B. hortorum* often round and within niches of tree-stumps; also the fine mould, rich in small litter particles. It would carry us too far to go into all details. Interesting observations have been made on the behaviour of the *B. pratorum* male perfuming spruce twigs with needles. While creeping on the upper side of the twigs the bumble-bee moves the unclasped proboscis like a paint-brush backwards and forwards along the needles. After such treatment the needles smell of the marker perfume.

The so-called passive perfuming probably depends basically on the habit of cleaning, which frequently occurs during active perfuming. When male bumble-bees are cleaning themselves from head to abdomen, the fur becomes smeared with the scenting products. The ventral side of the body from the hairs of the mandibles to those of the abdomen also becomes scented, when the bumble-bees are creeping along, perfuming. It is needless to say that the mouthparts are smeared with scenting products. Thus, from an early stage in their lives, bumble-bee males begin to function as veritable perfume-brushes. Chemical analyses confirm what is said above. It is not unexpected that bumble-bee males practically always smell of perfume, and that objects having served as rest- or sunbathing-platforms also smell of their perfume. Food flowers, as well as the females during copulation or copulatory attempts, also probably become perfumed by the males. In order to understand the importance of scent-stimulated behaviour and orientation movements in bumble-bees the circumstances briefly related here must be considered.

We have seen young females arriving at places suitable for sunbathing, and also observed copulation or copulatory attempts. The females do not seem to fly along flight paths as do the males. Only once have we seen the meeting of a female *B. pratorum* with a male in a flight path zone outside the sunbathing places (cf. Free, 1971). Only once, too, have we seen copulation on food flowers (*Ps. bohemicus*). We have observed that *B. lapidarius* may copulate in tree-crowns but also at the nest-entrance.

**LOCALIZATION OF SITE OF SECRETION PRODUCTION**

In our first publication regarding the chemical composition of the cephalic marking secretion of male bumble-bees (Bergström et al., 1968) we made a reference to the opinion posed by Haas
Table I. Capillary gas chromatographic analysis of excised mandibular and labial glands from males of the genera Bombus and Psithyrus

<table>
<thead>
<tr>
<th>Species</th>
<th>Gland</th>
<th>Analysis No.</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. hortorum L.</td>
<td>mandibular</td>
<td>502</td>
<td>1</td>
</tr>
<tr>
<td>B. hortorum</td>
<td>labial</td>
<td>503</td>
<td>1</td>
</tr>
<tr>
<td>2. B. agrorum F.</td>
<td>mandibular</td>
<td>504</td>
<td>1</td>
</tr>
<tr>
<td>B. agrorum</td>
<td>labial</td>
<td>505</td>
<td>1</td>
</tr>
<tr>
<td>3. B. terrestris L.</td>
<td>mandibular</td>
<td>502</td>
<td>1</td>
</tr>
<tr>
<td>B. terrestris</td>
<td>labial</td>
<td>503</td>
<td>1</td>
</tr>
<tr>
<td>B. terrestris</td>
<td>mandibular</td>
<td>504</td>
<td>1</td>
</tr>
<tr>
<td>4. B. lapidarius L.</td>
<td>mandibular</td>
<td>502</td>
<td>1</td>
</tr>
<tr>
<td>B. lapidarius</td>
<td>labial</td>
<td>503</td>
<td>1</td>
</tr>
<tr>
<td>Ps. rupesistris F.</td>
<td>mandibular</td>
<td>504</td>
<td>1</td>
</tr>
<tr>
<td>Ps. rupesistris</td>
<td>labial</td>
<td>505</td>
<td>1</td>
</tr>
<tr>
<td>Ps. rupesistris</td>
<td>mandibular</td>
<td>506</td>
<td>1</td>
</tr>
<tr>
<td>6. Ps. barbutellus K.</td>
<td>mandibular</td>
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<tr>
<td>Ps. barbutellus</td>
<td>labial</td>
<td>508</td>
<td>1</td>
</tr>
</tbody>
</table>

* Number of glands used in each particular analysis. In all analyses of labial glands only a portion of the glands were used.

Both preparations were placed directly in the splitter-free inlet system. Figs. 7 and 8 demonstrate that the main components identified in the cephalic secretion of male B. lapidarius, reported earlier (Kullenberg et al., 1970), emanate from the labial gland.

In order to confirm these findings 18 mandibular glands of Psithyrus rupesistris (see Kullenberg et al., 1970 for preliminary data concerning chemical composition) were extracted in diethyl ether. A portion of the extract, corresponding to 12 glands, was placed in the splitter-free intake system, giving rise to the gas chromatogram shown in Fig. 9 (analysis number 587). Even this analysis did not reveal any of the components present in the labial gland. For comparison, the capillary gas chromatogram (analysis number 523) shown in Fig. 10 may be studied. This emanates from a preparation of a portion of the labial gland.

The chromatograms shown in Figs. 7−10 are just four examples of a total of 19 analyses summarized in Table I. Eleven of these were analyses of mandibular glands, 8 of labial glands, representing 4 species of Bombus and 2 species of Psithyrus. The analyses of the labial glands from all species studied showed the presence in each species of relatively large amounts of the compounds characteristic of the particular marker secretion. The analyses of the mandibular glands, on the other hand, did not reveal the presence of these compounds. In the mandibular glands, the presence of minor quantities of other compounds, so far unidentified, has been noticed.

In connection with the previously mentioned observed behaviour of perfuming, superficial washings of the proboscis and the abdomen and thorax for 2 minutes in pentane were made in a few cases. Analyses by gas chromatography showed the presence of the characteristic compounds on these parts of the body. Fig. 11 shows a gas chromatogram of a washing of the proboscis from 7 individuals of B. lucorum L., "blonde form" (Bergström et al., 1973). The main compounds were identified by mass spectrometry. The results of tests on washing of the abdomen and thorax of B. terrestris males are given in Fig. 12. For comparison, a capillary gas chromatogram of a portion of a labial gland is shown in Fig. 13.

The deposition on leaves of Corylus avellana in the flight territories of nonadec-9-ene and all-trans-farnesol by B. hortorum males, was confirmed by
gas chromatography. These compounds are the main components in the marking secretion from this species.

ACKNOWLEDGEMENTS

This study has been supported by the Swedish Natural Science Research Council and the Axel and Margaret Ax:son Johnson Foundation. We are indebted to Dr Karl-Göran Bringer and Mrs Anna-Lis Kullenberg for patient and intelligent assistance in the field investigations and to Mr Lars Ahlquist, Miss Monica Appelgren, Miss Susanne Floberg, Mrs Inga Groth and Mr Jan Lundgren for skilful cooperation in the laboratory. The authors are indebted to Mr Rajesh Kumar for the linguistic revision of the manuscript.

BIBLIOGRAPHY

as the main component of the marking perfume of male bumble-bees of the species Bombus terrestris L. Arkiv Kemi 28: 31.


Stållberg-Stenhagen, S. 1972. Studies on natural odoriferous compounds. V. Splitter-free all glass intake system for glass capillary gas chromatography of volatile compounds from biological material. Chemica Scripta 2, 3.

Plate 1

1. Male *Psithyrus rupestris* scenting the edge of a leaf of *Corylus avellana*.
2. Male *Bombus lucorum* scenting small leaves.
3. Male *B. hortorum* scenting a hawthorn branch.
4. Male *Ps. campestris* scenting the upper side of a leaf.
5. Male *B. hortorum* scenting moss on bark.
6. Males of *B. hortorum* scenting bark of a stem-base.
7. Male *B. lucorum* inspecting a naturally scented leaf.
8. Male *B. lucorum* in ascending flight passing a naturally scented spot on a tree-stem.
9. Male *B. hortorum* in flight inspecting a scented spot at a stem-base niche.

Plate 2

Phenology of male bumble-bee scenting activity at a place of flight-path crossing and sunbathing, a *Corylus avellana* bush, in a sunny glade of a deciduous wood. The flights of the bumble-bees were observed during three summers. Every observed descent on the bush was marked with a coloured woollen thread or a dab of paint. Notice the increase with time in the number of colour-marks. Notice also the concentration of certain species to delimited parts of the leafage. Colour scale: red *Psithyrus campestris*, orange *Bombus lapidarius*, greenish *Ps. rupestris*, light blue *B. lucorum*, dark blue *Ps. bohemicus*, white *B. agroorum*.

3. End of the flying period, 20.8.1969. *Ps. rupestris* predominates over the entire leafage and the species has been numerous.
4. 11.8.1969. Three *Psithyrus* species have marked the bush. *Ps. rupestris* predominates. In the picture a male *Ps. rupestris* is seen approaching already-scented leaves and a male *Ps. bohemicus* is scenting a leaf which has previously been scented by both these species.
Field Experiments with Chemical Sexual Attractants on Aculeate Hymenoptera Males. II

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ABSTRACT

Kullenberg, B. (Entomological Department of the Zoological Institute, and Ecological Station of Uppsala University on Öland, Uppsala and Färjestaden respectively, Sweden.) Field experiments with chemical sexual attractants on aculeate Hymenoptera males. II. ZOON, Suppl. 1: 31–42, 1973

Several odoriferous compounds produced by the cephalic glands of male bumble-bees have been discovered. For solitary bees of the genus *Euceria* and the scolid wasp *Campyloscelis citata* it has been established by means of hexane extracts that the gland system of the head of the female produces male-attracting substances that function as sexual excitants. Apparently the male *Euceria* also exhalas male-stimulating substances. Volatile products from the gland system of the male bumble-bee head serve as territorial marker perfumes. Hexane extracts of heads of male bumble-bees function in the same way and, when applied to crude dummies of velvet, serve as stimulators of male copulatory activity. Certain synthetic compounds of the cephalic scenting secretion applied as the natural marker scents in the flight territories attract the flying males. Neither the *Euceria* extracts nor the extracts of heads of male bumble-bees are species-specific in their action.

Scents from the labella of *Ophrys* flowers function as stimulators of the male copulatory instinct of certain aculeate Hymenoptera. Hexane extracts of the labella have a similar influence on the Hymenoptera males. The *Ophrys* scents are specific for species groups and act specifically for species-groups with regard to the Hymenoptera males. Thin-layer chromatography fractions of *Ophrys* labella extracts show the strongest biological activity in alcoholic fractions.

INTRODUCTION

From the beginning of the field studies on the pollination of *Ophrys* L. and on the territorial perfume-marking by aculeate Hymenoptera males it was obvious that the problems involved have a common centre belonging to organic chemistry.

Before modern microchemical analytical methods were available, the author undertook preparatory field experiments with appropriate adequate natural products and objects.

As results from olfactory observations on scents of *Ophrys* flowers and aculeate Hymenoptera species were accumulated, it seemed worthwhile to try field experiments with so-called suspected structures. The results of these field experiments were reported in by the author in 1956 and 1961.

With the start of analytic chemical work in 1962, a considerable amount of documentation on volatile products with biological activity in Hymenoptera Aculeata has been compiled. Thus, with regard to bumble-bees (*Bombus* Latr. and *Psithyrus* Lep.) the chemical structures of several compounds constituting the marker perfumes of several species are now known (Kullenberg et al., 1970).

This paper sums up results from continued field experiments performed in connection with chemical work on the structures of biologically active, odoriferous compounds of Hymenoptera Aculeata and *Ophrys* orchids.

MATERIAL AND METHODS

All experiments reported here have been designed as choice experiments. In introductory experiments several substances have sometimes been tested together. This procedure always entails the risk of confusion of the dummies by the insects. Synergistic as well as masking or blocking effects may perhaps also influence the behaviour of the insects towards the baits. The choice experiment is therefore performed with one single scenting dummy and one without any scenting material, called the O-dummy.

In field experiments with biologically active substances one has, however, always to consider the risks of interferences of the kind mentioned, as it is
impossible to control naturally-occurring substances in the air of the experimental locality.

**Experiments with solitary bees**

In 1968 the scented dummy was just a rayon velvet piece of $11 \times 6$ mm (Plate 1, 12), but since 1969 it has been used together with an irregularly folded piece of green tulle as background in order to ensure uniformity of visual stimuli. Since 1971, the experiments—unless stated to the contrary—make use of the perfumed velvet dummy concealed within the piece of green tulle. Thus the observed guiding influence of the dark patch is eliminated (see Plate 1).

The distance between the dummies has generally been about 20 cm. It is important to locate the O-dummies so that they will not be swept by air loaded with scented material. Thus indirect comparisons may be made between scents. If nothing is said in the experimental reports about the O-dummy, it means that no marking has been observed towards it.

**Experiments with bumble-bees**

When studying the territorial marking behaviour of the bumble-bee males the scent compounds have been applied either on natural objects of the types frequented by the males themselves or on artificial objects made of wood, paper, etc. (see Plate 2, 1–6).

Crude dummies of black rayon velvet rolled up cylindrically, 20 mm in length and 10 mm in diameter, have been used when testing the influence of scents on male copulatory behaviour (see Plate 2, 7–8).

In general, two dummies were used in each experiment, as in experiments with solitary bees. The distance between dummies was, usually, about 20 cm but sometimes less.

**Evaluating the behaviour of insects towards scented baits**

When approaching the baits, the insects exhibit variable behaviour. This can be correlated to the ability of the scents exposed to excite and physiologically control the behaviour. If the natural behaviour of the Hymenoptera males provoked by scent stimulation from the insects themselves or from *Ophrys* flowers is taken as model behaviour, five types of behaviour may be discerned for solitary bees and four types for bumble-bees. These types of behaviour may be correlated to four degrees of excitation as demonstrated in Fig. 1. Bumble-bee males never, or at most in an indistinct way, perform “circling inspection” at close quarters of their own perfume marks or the experimental arrangements. Thus this behaviour type 3 may be omitted for bumble-bee males. The descent or visit to the dummies must be classified in two types: the quick visit or mere alighting and a more or less persistent visit with fairly easily characterised behaviour. Thus, solitary bee males may try to copulate with the tulle hiding the scented substratum, and bumble-bee males to sunbathe, perfume etc. For solitary bees I have judged the circling inspection and the quick visit to the bait as expressing the same degree of excitation. Circling inspection of a bait is said to occur when the flight route round the bait covers more than a third of the circumference.

**Test substances**

The flower and insect extracts were manufactured by putting 5 labella and 5 pieces of each of the three main parts of the Hymenopteron body in 1 ml n-hexane.

Three thin-layer chromatography fractions prepared from *Ophrys* labella extracts were chosen well separated from each other and dissolved in hexane. Fraction I contained mainly hydrocarbons, fraction II aldehydes, and for *O. bombyliflora* an unsaturated diterpene hydrocarbon in a relatively large amount, and finally fraction III aliphatic alcohols, together with sesquiterpene alcohols in larger amounts. The exact quantities of the different
compounds dissolved cannot be estimated. The concentrations of the volatile compounds used seem to fall within the limits of natural conditions.

For details regarding synthetic compounds used in bumble-bee tests, see p. 40. Fractionated natural material and synthetic material used in experiments with scoliids and solitary apids had a concentration of 10 mg in 1 ml hexane.

EXPERIMENTS

_Campsoscolia ciliata ciliata_ F.

*Introductory notes*

_Campsoscolia ciliata_, a scoliid wasp of the Mediterranean region, is the only effective pollinator of _Ophrys speculum_ Link. It is attracted, though definitely in a weaker way, to some other _Ophrys_ spp. The chemical analytic work indicates that the flower scent originating from the labellum may contain a tetra-cyclic sesquiterpene hydrocarbon that is longicylene-like in its molecular structure.

*Experiments with suspected structures*

A flower of _O. speculum_, presented visible or concealed for the vision, attracts regularly flying males. When in the same way as for _Eucera grisea_ males two preparative gas-chromatography fractions of copaene (called fraction I and II) and further a fraction of longicylene were exposed, seemingly weakly motivated approach flights were brought about, though not regularly.

*Experiments with hexane extracts of Ophrys labella and with extracts of thin-layer chromatography fractions*

The flying males were tested on four different days on the labellum-extract and the three extracts of the thin-layer chromatography fractions. As seen from Table I, where the results are summed up, the full extract and the thin-layer fraction III gave rise to the highest number of inspection flights of medium and high excitation degree, though all three fractions obviously exercised attraction.

*Experiments with extracts of female body parts*

These extracts were presented in the same localities as flower labellum extracts and in the same way during two days. The extract of the female abdomen was tested twice further. Without doubt the extracts of the heads and the thorax (Plate 1, 8) were the most attractive and caused the greatest degree of excitation.

_Eucera grisea_ F.

*Introductory notes*

The males of this species are attracted to the flower of _Ophrys bombyliflora_ Link and to less degree to those of _O. tenthredinifera_ Wild. In both cases the initiation of copulatory activity is obvious.

On Majorca this species has the same food plants to a large extent as _E. nigrilabris_.

*Experiments with suspected structures*

When tried with the same grapefruit materials as _E. nigrilabris_ in March 1968, _E. grisea_ males showed attraction behaviour during their copulation flight, though definitely to a much lower degree of excitation than _E. nigrilabris_. In 1969 the conditions seemed to be the reverse. In one single experiment (March 1969) _E. grisea_ males were tried with copaiba balsam dissolved in hexane and a column chromatography fraction of distilled copaiba balsam solution. The last substance contained a copaene isomer and probably a cadinene isomer. The _E. grisea_ males showed attraction behaviour, though without excitation.

When in 1971 _E. grisea_ males were tested on elemol and a fraction (called fraction II) from preparative gas-chromatography of copaene a very weak attraction could be supposed.

*Experiments with hexane extracts of Ophrys labella*

When tried on extracts of labella of _O. bombyliflora_ and _O. tenthredinifera_ the behaviour of the males reflects the difference in attractivity during natural conditions of the two _Ophrys_ species, that is to say, even if the same high degree (3) is obtained several times with both extracts, the extract of

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<tr>
<td><em>O. speculum</em> labellum extract</td>
<td>15</td>
<td>12</td>
<td>7</td>
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<tr>
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<tr>
<td>Fraction I</td>
<td>14</td>
<td>1</td>
<td>1</td>
<td>0</td>
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<tr>
<td>Fraction II</td>
<td>26</td>
<td>7</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Fraction III</td>
<td>16</td>
<td>12</td>
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O. bombyliiflora seems to be much more effective (see Plate 1, 1).

Experiments with thin-layer chromatography fractions

O. bombyliiflora is regularly pollinated by the male E. grisea. When tested in the same way as the male E. nigrilabris on O. bombyliiflora labelia extract and the three thin-layer chromatography fractions respectively, it was established that next to the complete extract the alcohol fraction demonstrated the highest attractivity.

Eucera nigrilabris Lep.

Introductory notes

It was stated by the author as a result of field observations in Morocco and on Majorca that the flowers of O. tentredinifera exhaled a perfume acting as a sexual excitant on the male E. nigrilabris and provoking it to make approach flights to, and copulation attempts with, the labelia of the flowers. The scent of the flower was found to be farnesolic, but also to possess a scent of the type of that of gayol or an empty cigar-box. This last-mentioned scent note seems to be more pronounced in O. scopolax Cav. which has a homologous floral ecology (Kullenberg, 1961). Anyhow, the scent of the flower is rather variable. Finally, this last-named scent note was found to resemble that of copaene. In the labelia of O. tentredinifera and O. scopolax, and probably O. apifera Huds., may occur a regular tri-cyclic sesquiterpene hydrocarbon (copaene-like).

Experiments with suspected structures

1. A column-chromatography fraction from an extract of grated peel of grapefruit called fraction I (the fifth and last fraction) was tried together with two other fractions called II and III. The scenting material was dissolved (10 mg per ml) in hexane. The substances were applied to black velvet pieces of the type mentioned above. The fraction I contained mono- and sesquiterpenes. The sesquiterpenes were copaene, ylangene and a cadinene. This fraction I had a scent close to that of the flower of O. tentredinifera and O. scopolax.

The experiments were performed in March 1968 during two days in a locality where E. nigrilabris males accomplished their territorial- and copulation flights. From these introductory experiments it was quite clear that fraction I exercised attraction and a certain excitation on the males to copulatory activity (Plate 1, 12). Fraction II provoked some approach flights, whereas fraction III did not seem to be active.

2. Fraction I was exposed on a following day in the same way together with a concealed flower of O. tentredinifera, and on another day together with one concealed flower of O. tentredinifera and O. bombyliiflora respectively. It was obvious in these experiments that the scent of the flower of O. tentredinifera dominated the scent of the terpene loaded fraction I.

3. In March 1969, experiments with the grapefruit substances of 1968 were continued in the same locality on Majorca. Anyhow, though clearly attractive to the males of E. nigrilabris as well as to E. grisea this year too, this so-called grapefruit fraction I now showed a comparatively weak attraction power on E. nigrilabris. In fact E. grisea males seemed to be somewhat more attracted by the substance. These experiments have not been continued, as it seems adequate to proceed with the chemical work before recommencing field experiments of this type.

4. In the same localities as for experiments 1–3, γ-cadinene and elemol, among other substances, were tried in 1971. They did not seem to exercise any sex attractant function on E. nigrilabris males, but both compounds might be supposed to be weakly attractive.

Experiments with hexane extracts of Ophrys labelia

In these experiments performed on Majorca in spring 1972, extracts of the Ophrys species indicated in Tables II and III were tested (Cf. Plate 1, 3). The results from the different tests are quantitatively uneven because of weather conditions. Nevertheless they support the results obtained from field observations. Regarding the highest degree of excitation achieved, no differences were to be found between O. arachnitiformis Gren. et Phil. and the three other typically Eucera-pollinated species. Anyhow, the extract of O. arachnitiformis did not attract in the same regular way as the other extracts. The highest degree of excitation ever achieved, and the excitation degree of highest frequency summed up during all experiments are the same, namely degree 3.
than the attraction of the dissolved fractions. Nevertheless, it seems clear that all three fractions of both Ophrys species have attractive ability, but that the alcoholic fraction provokes the highest number of approach flights and, like the full extracts, even relatively more of the activity called circling inspection (excitation degree 3) (see Plate 1, 2).

**Eucera tuberculata F.**

**Experiments with hexane extracts of Ophrys labella**

Under natural conditions this species is chemically attracted to Ophrys flowers in the same way as the preceding and the following species. Depending on weather conditions the tests could not be performed as well-planned as for the other Eucera spp mentioned here. Nevertheless the results in the table (Tables II and III) agree with the results obtained from field observations on visits of E. tuberculata to the flowers of the Ophrys spp. in question.

**Eucera longicornis L.**

**Experiments with hexane extracts of Ophrys labella**

Experiments performed during the same days with flower extracts of four Ophrys spp. confirm the lesser degree of attractivity of O. bertolonii Mor. (Table II) though even the extract of this species may provoke a high degree of excitation (Table III). When tested together during the same six days, extracts of O. tenthredinifera and O. bombyliflora could not be differentiated regarding excitation ability. It deserves to be mentioned that E. longicornis males are the only ones of the Eucera spp. hitherto tried that have performed regularly attempted copulation with the piece of tulle hiding the perfumed velvet piece. This behaviour could be observed without difficulty for extract of O. tenthredinifera as well as for that of O. bombyliflora. E. longicornis seemingly has a lesser specific releaser mechanism for tactiley stimulated movements than E. grisea and E. nigrilabris (Cf. Plate 1, 4 and 6).

**Experiments with thin-layer chromatography fractions**

Fractions from O. tenthredinifera extracts as well as of O. bombyliflora extracts have been tried. Every experimental series of a day was begun by testing with the full extracts of each Ophrys species. It must be emphasized that the concentration of volatile products was higher in the full extract than in each of the thin-layer chromatography fractions extracted in hexane. The attraction of the full extract was in both cases obviously stronger
Table IV. Comparative testing of the attractiveness of extracts and three thin-layer chromatography fractions of labella extracts of *O. tentheadinifera* and *O. bombylliflora* respectively to the male *E. longicornis*

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<td>Behaviour type ...</td>
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</table>

A. *O. tentheadinifera* labelle
 extract
Thin-layer chromatography
Fraction I 11 6 1 0 0
Fraction II 8 6 2 0 0
Fraction III 4 9 6 1 5

B. *O. bombylliflora* labelle
 extract
Thin-layer chromatography
Fraction I 3 5 0 0 0
Fraction II 9 6 1 0 0
Fraction III 8 8 1 0 1

from one single day (summer 1972) with adequate weather conditions and consequently high motivation on the part of the males to react (Table IV).

The experiment comprises volatile substances of *O. tentheadinifera* and *O. bombylliflora* labella.

Even for the male *E. longicornis* the alcoholic fractions are the most attractive ones. The sound physiological condition of the experimental population on the day of the experiment is indicated by the high frequencies of high-excitation degree behaviour towards the full extracts.

**E. longicornis** and **E. grisea**

**Experiments with extracts of the main body parts of the female**

*E. grisea* and the whole body of the male *E. tuberculata*

Hexane extracts of the female *E. grisea*, head, thorax, abdomen and head + abdomen, and of the male *E. tuberculata*, whole body, were exposed in the flight territories. The extracts of all body parts of the female *E. grisea* have a full sexual exciting effect on the male *E. longicornis* as well as on the male *E. grisea*, but even the extract of the male *E. tuberculata* is clearly of sex pheromone function towards the male *E. longicornis*. These results will be discussed together with the results from the experiments with bumble-bee male cephalic secretion. Thus copulation-stimulating secretions for males do not seem to be species-specific and not even necessarily produced exclusively in the female body (see Plate 1, 5, 7, 9–11).

**Bombus Latr. and Psithyrus Lep.**

**Introductory remarks and experimental arrangements**

In the present report the author sums up some preliminary experiments, designed to investigate the behaviour of the bumble-bee males in their flying territories in the presence of their own perfumes or of synthetic material found in bumble-bee male perfumes. These studies imply that the scenting materials have been placed by the experimenter in definite locations, and that, at the same time, all natural bumble-bee markings in the vicinity of the experimental arrangements have, as far as possible, been removed.

The experimental scent materials were applied either to natural objects such as fresh or withered leaves, grass-stems, branches, bark or pieces of bark, or to artificial objects made of paper or wood in the shape of leaves or tree-stumps (see Plate 2, 1–6). Dummies made of velvet have been used, for the study of the copulation behaviour of the males.

The natural behaviour of the male bumble-bee when applying scent substance to different objects is described in a paper by Kullenberg et al. (1973). The flight paths of different species are treated in a paper by Bringer (1973).

A few definitions will be presented in order to make intelligible certain concepts used by the present author.

The **flight paths** of the bumble-bee males are, as a rule, individual. The directions of the flight paths are not, however, really fixed in the terrain, each individual having its own flight path zone. As parts of the flight paths of several individuals may lie close to each other, the flight path zones probably overlap to some extent, being used by more than one individual, not necessarily of the same species. The flight paths build up a three dimensional net work in the **flight territory** of the males into which the young females arrive, though not perfuming in the way the males do. In the flight territories the perfumed points or the restricted perfumed areas act as "scenting lighthouses". It is clear that the flights are essentially guided by sight. Different species seem to express somewhat different preferences regarding topography and vegetation structures, though the ability of the species to ethological adjustment seems quite considerable.

When performing field experiments, the choice of objects for the application of the scenting
substances in the introductory phase of the research must be made against the background of what is known about the natural perfuming habits.

Thus, for example, as the male _B. hortorum_ regularly perfumes tree-trunk bases or tree-stumps among other things it is enough to apply test substances on dummies representing tree-stumps. The placing of dummies representing environmental objects must be done carefully. The male _B. hortorum_, in my experimental locality has not accepted a new artificial perfume substratum too near a natural one already involved in the territorial flying activity. I have found that a new perfume-marking object must be placed at least 3 m from an old one to be accepted. It seems that an object appropriate for sunbathing can relatively easily be accepted as an environmental element of the male flight territory (Plate 2, 1).

The scent tests briefly summed up here were performed in the summers of 1969–1972, and for all species except _B. lucorum_ in the glade of a deciduous wood. This glade is a traffic point for flying bumble-bee males of several species and offers excellent sunbathing places. The wood and its surroundings offer good nest sites. _B. lucorum_ males were studied in two localities of shady young deciduous woods—a typical environment of the species for its territorial flight.

The extracts of bumble-bee heads were prepared in the following way. For the experiments of 1969, 10 heads were extracted in 1 ml hexane, and to this extract was added 1 ml of a saturated silicon solution in hexane. Later the number of heads was fixed at five per ml and no silicon solution was added. Synthetic materials were also dissolved in hexane. Unless stated to the contrary, the concentration for most experiments was fixed at 1:200. To obtain this concentration the basic solution was prepared with an equivalent quantity of saturated silicon solution in hexane. This heightens the durability of the test material. The test substances were applied to the dummies with the aid of a glass stopper taking about 10 μl and used as a stamp. During the years a different number of test substance stamps have been used as perfuming standard: 1969 20, 1970 10, 1971 and 1972 5. The variation in quantity used in the experiments does not seem to have had any influence on the behaviour.

The experiments have almost always been arranged as choice experiments with dummies in pairs, one scented and one non-scented. On the large tree-stump dummy two scent compounds and one non-scented silicon solution have been used simultaneously. The non-scented dummy and non-scented test substance are called O-dummy and O-substance respectively.

When studying the male copulatory behaviour the dummies were immediately changed after having been touched by a bumble-bee male. It has been stated that the visits of a bumble-bee—even instantaneous—to a dummy contaminate it with natural male scenting products (see Plate 2, 7 and 8).

_B. hortorum_ L.

All-trans-farnesol is the main component of the marking perfume (Kullenberg et al., 1970). Synthetic trans-farnesol applied to a tree-stump dummy made of brown paper showed attractivity, but when tested comparatively with ethyl dodecanoate—the main odoriferous component of the marking secretion of the "dark form" of _B. lucorum—even this compound exercised attraction. Synthetic trans-farnesol in a concentration of 1:2 000 applied to dummy leaves made from green blotting paper provoked inspection flights and perfuming of the dummy leaves (Plate 2, 4). The minor secretion component geranylgeraniol also attracts.

_B. lucorum_ L.

1. Extract of male heads ("blonde form" of the species, cf. Bergström et al., 1973) applied on velvet bumble-bee dummies was used in testing the ability of the male cephalic secretion to initiate copulatory activity. The experiments were performed in a male flying territory. A scented and an O-dummy were fixed to tree stems, about 2.5 m above the ground, standing in the flight path zones. The experiment was performed over three days, 3 h 35 min, 1 h 55 min and 2 h 25 min each day, respectively.

The scented dummy provoked inspection behaviour of all degrees of excitation, as well as some copulatory attempts each day. The O-dummy was tried in this last-mentioned way by one male only once, and very quickly at that. On the third day the O-dummy received almost as many inspection approaches and visits as the scented one, but only two of a high degree of excitation, whereas the scented one received seven.

2. One day the _B. lucorum_ males in the same locality and in the same way were tested on extract
of *Ps. bohemicus* male heads applied to one of two velvet dummies. The activity towards the scented dummy and the O-dummy was now quite equal, indicating comparative weakness in attractive quality of the part of *Ps. bohemicus* substance.

The following day the *B. lucorum* males were tested three times, 15 min each time, on extracts of male head secretion in this way: (A) *Ps. bohemicus*, (B) *B. lucorum*, and (C) *Ps. bohemicus*. The results in Table V compiled from the field notes, show a difference in influence from the two types of bumblebee perfumes. It must be pointed out in this connection that *Ps. bohemicus* is observed as a so-called nest-parasite, that is to say, a symbiotic partner of *B. lucorum* (Bischoff, 1927; Hammer & Nørgaard Holm, 1970; Knechtel, 1955; Step, 1932). It may be mentioned that the shady parts of the flying territory do not seem to be the normal copulation habitat of *B. lucorum* (Plate 2, 7).

3. When *B. lucorum* male head extract was used experimentally in the same way in a sunny locality where *Ps. campestris* of both sexes occurred, *Ps. campestris* males were attracted to the *B. lucorum* perfumed dummies and even eagerly performed copulatory attempts with them (Plate 2, 8).

### Table V

<table>
<thead>
<tr>
<th>Time records</th>
<th>Scented dummy</th>
<th>1) Excitation degree 2) Behaviour type</th>
<th>Non-scented dummy</th>
<th>1) Excitation degree 2) Behaviour type</th>
<th>Passages without specific reaction</th>
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</thead>
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<td></td>
<td>1)</td>
<td>2)</td>
<td>3</td>
<td>3c</td>
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<tr>
<td><strong>A. Ps. bohemicus</strong></td>
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<tr>
<td>scent</td>
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<tr>
<td>08.10-08.25</td>
<td>1+</td>
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<td>08.11</td>
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<td>08.16</td>
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<td>08.23</td>
<td>1+</td>
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<tr>
<td><strong>B. B. lucorum f. &quot;medium blonde&quot; scent</strong></td>
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<td>08.26-08.41</td>
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<td>08.26.30</td>
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<td>08.27</td>
<td>1+</td>
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<td>08.29</td>
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<td>08.41</td>
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<td><strong>C. Ps. bohemicus</strong></td>
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<tr>
<td>scent</td>
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<td>08.48-09.03</td>
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<td>1+</td>
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<tr>
<td>09.01</td>
<td>1+</td>
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</tbody>
</table>

Sometimes during the field experiments the distance from the dummy at which the inspecting male stops and performs the final activity are estimated with the following symbols in the field minutes: 1+ 0–5 cm, 2+ 5–10 cm, 3+ 10–20 cm, 4+ 20–30 cm, c copulation attempt. The stop distance apparently does not depend primarily on the physiological suitability of the structure but on air current turbulence and wind force. The behaviour type 3 means here descent to the dummy.
B. lapidarius L.

B. lapidarius and Ps. rupestris form a symbiotic unit as the latter species is a nest-parasite on the former. At times, the males of the two species perform their territorial flying together. When studying the perfuming behaviour of Ps. rupestris on two experimental days I have seen B. lapidarius workers approach dummy leaves scented with 1-geranylgeraniol and geranylecitronellol, the last-mentioned compound being the main component of the marking secretion of the male Ps. rupestris. It was even observed that a B. lapidarius worker may descend on such a dummy leaf scented with geranylecitronellol (Plate 2, 6).

Ps. bohemicus Seidl

1. In the summer of 1969 hazel leaves perfumed by Ps. bohemicus males were bundled together (5–7) and applied as scenting device in flight-path zones of the species and these baits immediately attracted the flying males and provoked flying inspections and descents with perfuming either on the bundled leaves or on leaves in the vicinity. Such naturally-perfumed leaves keep the stimulation ability for at least 24 hours.

Extract of Ps. bohemicus male heads applied on hazel leaves provoked the same behaviour.

2. The tests of the male Ps. bohemicus reactions on chemical stimuli were continued with synthetic compounds in 1969 and 1970. On fresh hazel leaves was applied citronellol solution in concentrations of 1:200, 1:2000 and 1:20000 with the result that the males arrived on inspection flights at close quarters. The preparation of 1:200 stimulated the males to descend on the scenting leaves (Plate 2, 3). Citronellol is a component of the marking secretion of the species.

It was also found that the Ps. bohemicus males were attracted to geraniol and dihydrofarnesol, when these compounds were presented to the flying bumble-bees in the same way.

3. Some experiments were performed in the summer of 1972 in order to study the reactions of the male Ps. bohemicus regarding its copulatory behaviour. Extract of heads of the male Ps. bohemicus was applied to velvet dummies. The males were seen to perform copulatory attempt, with the scented dummies. The males Ps. campestris were also excited to copulatory attempts, by means of the olfactory stimulation of Ps. bohemicus male head extract.

Ps. rupestris F.

1. In experiments in the summer of 1969, extracts of heads of Ps. rupestris and B. lapidarius males were applied to live hazel leaves and to dummy leaves of green blotting paper respectively in the flight territory of the former species. The males of Ps. rupestris displayed attraction behaviour to both kinds of extracts (Plate 2, 5), but obviously the species own substances had the stronger influence and brought about alighting on the leaves. Even extract of the male Ps. bohemicus was attractive for the male Ps. rupestris.

2. In the summer of 1969 it was observed that males of Ps. rupestris in the territorial flight were attracted by geraniol and dihydrofarnesol. A preliminary assumption later made was that geranylgeraniol and geranillylinalool might be attractants in the flight territory, and as it was known that geranylecitronellol is the main component of the marking secretion of the species, this substance was tested in the field in 1971. Applied to dummy leaves of blotting paper the geranylecitronellol could be seen to exercise a rather strong attractive influence on the male Ps. rupestris during its daily routine flying. It provoked inspection approaches and descents. When geranylgeraniol was tried once again it seemed probable that it exercised some attraction.

Ps. campestris Pz.

This species of Psithyrus has hitherto been observed in experiments only occasionally. Anyhow, it was seen that the male Ps. campestris approached bunched leaves with naturally delivered perfume of the male Ps. bohemicus. When velvet dummies, perfumed with extract of the male B. lucorum, together with O-dummies, were on one occasion put in a flight path zone also used by Ps. campestris, it was twice observed that Ps. campestris males after approach flying, eagerly tried to copulate with the B. lucorum perfumed dummies (Plate 2, 8). The Ps. campestris males tried copulation twice, though not so eagerly as in the first case, with the O-dummy, fixed as usual at a certain distance from the scented one. After both descents on the O-dummy it was easy to observe that the latter acquired the scent of the male Ps. campestris.
Table VI

<table>
<thead>
<tr>
<th>Natural occurrence of the compounds studied</th>
<th>Compounds in synthetic form</th>
<th>Male bumble-bees attracted</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. sporadicus</td>
<td>ethyl n-dodecanoate</td>
<td>B. hortorum</td>
</tr>
<tr>
<td>B. lucorum “dark”</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. lucorum “blonde”</td>
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<td></td>
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<tr>
<td>B. terrestris</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. patagiatus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. praetorius</td>
<td>citronellol</td>
<td>Ps. bohemicus</td>
</tr>
<tr>
<td>Ps. bohemicus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. cullumanus</td>
<td>geraniol</td>
<td>Ps. bohemicus</td>
</tr>
<tr>
<td>B. praetorius</td>
<td></td>
<td>Ps. rupestris</td>
</tr>
<tr>
<td>B. hortorum</td>
<td>all-trans-farnesol</td>
<td>B. hortorum</td>
</tr>
<tr>
<td>B. praetorius</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ps. barbatiellus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. terrestris</td>
<td>2,3-dihydrofarnesol</td>
<td>Ps. bohemicus</td>
</tr>
<tr>
<td>B. terrestris</td>
<td></td>
<td>Ps. rupestris</td>
</tr>
<tr>
<td>B. hypnorum</td>
<td>geranylgeraniol</td>
<td>Ps. rupestris</td>
</tr>
<tr>
<td>Ps. rupestris</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. hortorum</td>
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<td></td>
</tr>
<tr>
<td>B. subterraneus</td>
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<td>B. lucorum “dark”</td>
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</tr>
<tr>
<td>B. cullumanus</td>
<td>geranylgeraniol</td>
<td>B. hortorum</td>
</tr>
<tr>
<td>B. praetorius</td>
<td></td>
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</tbody>
</table>

SUMMARY

This paper reports the results of field experimental work on chemical sexual attraction on the male of certain aculeate Hymenoptera. In view of the connection between these studies and the theory of evolution, biocenology and the study of isolation between species, some summarising conclusions and general points of view may be forwarded simply to outline planning of future experimental work.

1. Hexane extract of heads of the female Eucera grisea attracts and sexually excites not only the male of this species itself but also the male E. longicornis. Even extracts of the female thorax and abdomen are exciting in the same way though apparently more weakly. The influence of these extracts upon the male Eucera seems to parallel that of the scent of the flower of O. bombyliflora or that of the extract of its labellum. The attractiveness of the extracts of the female thorax and abdomen may be due to contamination from the cephalic secretions delivered through the mouthparts. These scentings secretions must be dispersed through the hair-coat by the cleaning activity. It is intended by means of future work to try to find out if sexual pheromones emanate from abdominal glands and if perhaps synergistic effects from cephalic and abdominal secretions may be responsible for the attainment of copulation within the species.

As extract of the body of the male E. tuberculata sexually excites the male E. longicornis it seems reasonable to suppose that as in bumble-bees (Bombus and Psithyrus) even the male Eucera produces substances stimulating the male copulation behaviour. Extracts of head and thorax of the female Campsoscolia ciliata were sexually exciting for the male of this species.

2. The scents of the Ophrys flowers contain a series of volatile compounds of varying structures. In all Ophrys species hitherto studied the alcoholic fractions of thin-layer chromatography preparations of extracts of the labella have exercised stronger sexual excitation on the pollinating male Hymenoptera (Gorytes, Campsoscolia, Andrena, Eucera) than other fractions.

In their stimulating ability upon Eucera males,
Ophrys flower scents are species-group specific. This circumstance is more pronounced when Eucera males are presented an extract of five labella/1 ml hexane than when they are presented one single live labelllum.

3. Extracts of male Bombus and Psithyrus heads function in the same way as marker perfumes delivered from the cephalic glands—that is to say, as territorial flight stimulators and copulation excitants, when applied to velvet dummies where they act as sexual pheromones. Neither of these chemical stimulating functions is species-specific. The biocoenotic and symbiotic (nest–parasite–host relations) functions of the marker perfumes appear conceivable.

The ability to attract beyond the limits of the species is characteristic even of individual compounds in the marker perfumes, as the presentation of results from field experiments shows (Table VI). The table sums up the results hitherto obtained in the experiments with the stimulation by defined chemical structures from the cephalic scenting secretions.

4. It is extremely important to recognize that the organic molecule is the primary level of organization of life. This implies that in scientific work concerned with this level of organization, the study of form and of function are inextricably joined. In biological problems, as, for example, those dealing with biological signals where molecular activity is concerned, the analytic chemical work is of fundamental importance. It is evident that all research on the function of biologically active substances demands analysis of molecular structure as exactly as possible.

ACKNOWLEDGEMENTS

This study has been supported by the Swedish Natural Science Research Council and the Axel and Margaret Axson Johnson Foundation. My gratitude is due to Mr. Lars Ahquist, Miss Monica Appelgren, Miss Susanne Floberg and Mrs. Inga Groth for skilful assistance in manufacturing test materials. I am indebted to my wife, Anna-Lis Kullenberg, and to Miss Birgitta Carlberg for assistance in the field experiments. In this case, as in many others, I wish to express my warm gratitude to Miss Gunnel Wällén for preparing the manuscript and to Mrs. Inga Thomasson for arranging the illustrations. I am indebted to Mr. Rajesh Kumar for the linguistic revision of the manuscript.

BIBLIOGRAPHY


Vecht, J. van der. 1928. Fauna van Nederland, aflevering 4, Hymenoptera Anthophila (Q XIII m) A. Andreana. Leiden.

Plate 1
1. Male Eucera grisea approaching from below the extract of Ophrys bombyliflora labella during sunny weather with practically no wind and moderate temperature.
3. Male E. nigrilabris approaching from above extract of O. tenellulinifera During sunny, warm weather with ascending air.
4. Male E. longicornis in copulatory attempt with an inflorescence of Plantago during scent stimulation from extract of O. tenellulinifera labella.
5. Male E. longicornis in copulatory attack against a withered spot on a leaf. Scent stimulation from female E. grisea head extract.
8. Male Campsocosta ciliata approaching female thorax extract.
11. Male E. longicornis copulatory attempt with tulle. Scent stimulation from male E. tuberculata body extract.
12. Male E. nigrilabris copulatory attack on a velvet dummy prepared with a fraction of an extract of grapefruit peel.

Plate 2
1. Male Bombus hortorum sunbathing on a bumble-bee hive.
2. Male Psthyrus campestris approaching a leaf with the species’ own scent (red mark) and bundled leaves with Ps. bohemicus scent.
4. Male B. hortorum scenting dummy leaves prepared with trans-farnesol solution 1:2 000.
5. Male Ps. rupestris approaching a dummy leaf prepared with male B. lapidarius head extract.
7. Male B. lucorum in copulatory attempt with velvet dummy prepared with male B. lucorum head extract.
8. Male Ps. campestris in copulatory attempt with a dummy prepared with male B. lucorum head extract.
Reaktionen von Riechrezeptoren männlicher Solitärbienen (Hymenoptera, Apoidea) auf Inhaltsstoffe von Ophrys-Blüten

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ABSTRACT

Priesner, E. (Max-Planck-Institut für Verhaltensphysiologie, Seewiesen, Deutsche Bundesrepublik.) Reaktionen von Riechrezeptoren männlicher Solitärbienen (Hymenoptera, Apoidea) auf Inhaltsstoffe von Ophrys-Blüten. [Reactions of olfactory receptors in male solitary bees (Hym., Apoidea) to fragrance components of Ophrys flowers.]
ZOON, Suppl. 1: 43–54, 1973

The specific attractivities of Ophrys fragrances for males of certain aculeate Hymenoptera (Kullenberg, 1961) have been confirmed by electrophysiological recordings. Extracts were prepared from the labela of 18 Ophrys forms pertaining to 11 species. The effects of the extract on antennal receptors of about 50 species of bees were determined quantitatively using the electroantennogram (EAG) technique. Ophrys lutea, O. sphecodes ssp. sphecodes, O. arachnitiformis, O. tenthredinifera, O. scolopax, O. bombyliflora, and O. apifera elicited high EAG responses in certain species, whereas the remaining Ophrys forms did not display high responses in any of the species investigated. The outstanding electrophysiological activity of O. lutea was found to be restricted to male Andrena pubescens; the respective activities of O. sphecodes ssp. sphecodes and O. arachnitiformis were limited to male Andrena jacobi; and those of O. tenthredinifera, O. scolopax, O. bombyliflora, and O. apifera to male Eucera tuberculata and E. longicornis. There were weak or no EAG reactions to the 18 Ophrys types in the males of 22 additional species of Andrena, in the males of 11 additional genera of Apoidea, and in the females of all the test species. The EAG patterns to the flower extracts (Fig. 2) closely resembled the groups of Ophrys species and pollinators established on base of their attraction specificity by Kullenberg (1961). These patterns were employed for discussing the distribution of identical or similar fragrance components among the 18 Ophrys forms. EAG responses to chemically defined components as well as to various derivatives were determined using the experimental design applied to the flower extracts. \( \gamma \)-Cadinene, assumed to be the main terpenoid component in the labelum extract from several Ophrys species (G. Bergström, E. and S. Stenhagen, unpublished data), elicited extremely high EAGs on males of Eucera tuberculata and E. longicornis. Other Cadinene as well as Amorphenes, Muuroenes, Bulgarenes, Copaenes, Ylangenes (Fig. 3), structurally different sesquiterpenes, and sesquiterpene alcohols were at least \( 10^3 \) times less effective than \( \gamma \)-Cadinene. The EAG response pattern to \( \gamma \)-Cadinene roughly parallels the respective pattern to labelum extracts of Ophrys species known to be strongly attractive for Eucera males.

EINLEITUNG

olfaktorische Signale der Ophrys-Blüte deren nicht ganz adäquates taktiles Reizmuster zu kompensieren scheinen. Wenngleich diese Untersuchungen noch keinen Aufschluß über die chemische Struktur der beteiligten Substanzen brachten, lassen sie keinen Zweifel, daß Ophrys-Arten Stoffe produzieren, die mit dem weiblichen Sexualpheromon bestimmter Hymenopteren-Arten entweder identisch sind oder dessen Wirkung imitieren.


Die Untersuchungen erfolgten in enger Zusammenarbeit mit Prof. B. Kullenberg (Upsala), Prof. E. und S. Stenhagen (Göteborg) und Dr. G. Bergström (Göteborg). Die Labellinextrakte der Ophrys-Arten sammelte mit wenigen Ausnahmen Bertil Kullenberg in Südfrankreich und auf Mallorca. Ihm und Gunnar Bergström verdanke ich auch zahlreiche biologische und chemische Angaben. Dünnfischfraktionen von Ophrys-Extrakten, Testfraktionen ätherischer Öle sowie eine größere Zahl reiner Substanzen wurden aus Göteborg, eine Serie weiterer Sesquiterpenkohlenwasserstoffe durch die Firma Dragoco (Holzminden, Dr. E. Klein) zur Verfügung gestellt.

DIE UNTERSUCHTEN HYMENOPTEREN-ARTEN

Bei der Wahl der Versuchstiere interessierten in erster Linie Hymenopteren-Gattungen, aus denen spezifische Ophrys-Pollinatoren beschrieben sind. Es sind dies die Gattungen Campsoscelia (Scoliidae), Gorytes (Sphecidae), und Colletes, Andrena, Eucer a und Tetralonia (Apidae). Von Campsoscelia und Tetralonia stand noch kein Lebendmaterial zur Verfügung; von Gorytes und Colletes bisher nur andere Arten als die Ophrys-Pollinatoren G. campestris L., G. mystaceus L. und C. cucurullus L. Nachfolgende Andrena- und Eucer-Arten wurden untersucht:

Andrena (Nomenklatur nach Stoeckhert 1954, Untergattungen nach Warncke 1968)
1. pubescens Ol. (= nitida Geoffr.), 2. nigroanea K. (Subgenus Melandrena);
3. carbonaria L. ssp. nigropinosa Thoms. (Subg. Plastandrena);
4. haemorhoa F. (= albicans auct.) (Subg. Bioreolina);
5. flovipes Pz., 6. gracida Imh. (Subg. Zonandrena);
7. wilkella K., 8. labry Allk. (Subg. Tennandrena);
9. bicolor F. (= gynanana K.), 10. fulvida Schck. (Subg. Euandrena);
11. propina Schck., 12. dorsata K. (Subg. Simandrena);
13. jacobii Perk. (= trimmerana auct. nec K.) (Subg. Hoplandrena);
14. chrysosceles K. (Subg. Notandrena);
15. ventralis Imh. (Subg. Parandrena);
16. denticulata K., 17. fuscipes K. (Subg. Chernidandrena);
23. minuta L., 24. floridona Ev. (Subg. Micrandrena); sowie eine Reihe noch nicht determinierter weiterer Arten vor allem der minuta- und der ovata-Gruppe.

Eucer. 1. tuberculata F. 2. longicornis L.

Aus 11 weiteren Bienengattungen, die später genannt werden, wurden einzelne Arten in die Tests einbezogen. Alle Versuchstiere stammen von süddeutschen Fundstellen (Maintal bei Würzburg; Altmühltal bei Eichstadt und Riedenburg; Botanischer Garten München; Umgebung Starnberg und Seewiesen; Puplinger Au bei Wolfratshausen).
DIE UNTERSUCHTN OPHRYS-ARTEN

Eingehender untersucht wurden 18 Ophrys-Formen, die sich auf 11 Arten des Systems von Nelson (1962) verteilen:

1. insectifera L.
2. speculum Link
3. lutea Cav.
4a. fisca Link, Form I („versus ssp. omegafera“)
4b. Form II („Labellen klein, schmal gelb gerandet, deutliche Basalfurchen, Normaltypus“)
4c. Form III („Labellen ziemlich lang u. dick, deutliche Basalfurchen“)
5. berioloni Mor.
6a. sphaecodes Mill. ssp. sphaecodes Mill.
6b. ssp. atrata Mayer
6c. ssp. litigiosa Bech.
7a. arachnitiformis Gren. et Phil., Form I („rosa Sepalen“)
7b. Form II („weisse Sepalen“)
7c. Form III („grüne Sepalen, Spiegelzacken ungegliedert“)
7d. Form IV („grüne Sepalen, Spiegelzacken gegliedert“)
(7c und 7d „versus sphaecodes provincialis“)
8. tentredinifera Wild.
9. scolopax Cav.
10. bombyliflora Link
11. opifera Huds.


VERSUCHSTECHNIK

Zur Wirksamkeitsbestimmung im elektrophysiologischen Test mußten von den Blütenextrakten, Weibchenextrakten, Fraktionen und reinen Substanzen zunächst Reizquellen bekannter Menge hergestellt werden. Dazu wurde über mehrere Zehnnerstufen in n-Hexan verdünnt und Lösungsmengen bekannter Konzentration auf Filterpapiere 14 × 14 mm aufgebracht, die nach Abdampfen des Lösungsmittels die Reizquellen ergaben. Der Labellenextrakt jeder der 18 Ophrys-Formen wurde als Menge von 0,01, 0,1 und 0,5 Labellum geprüft. In analoger Weise wurden von Abdominal- und Kopfextrakten von Weibchen 0,01-, 0,1- und 0,5-Äquivalente hergestellt. Bei den reinen Testsubstanzen lagen die Reizquellenmengen zwischen 0,01 μg und 10 μg.

ERGEBNISSE UND DISKUSSION

An jeder der genannten Andrena- und Eucera-Arten wurde die Wirksamkeit der Labellenextrakte der 18 Ophys-Formen im Elektroantennogramm (EAG) quantitativ bestimmt. Diese Bestimmungen erfolgten stets in aufsteigender Konzentration, d. h. zunächst wurden an einem Antennenpräparat alle Ophys-Formen als 0.01- bis 0.1- und schließlich als 0.5-Labelllumäquivalent geprüft. Alle Aussagen beziehen sich auf die während der Reizsekunde registrierte EAG-Amplitude (Abb. 1).


Abb. 1. Elektroantennogramm-Registrierungen an Bienemännchen (Andrena, Eucera) bei Reizung mit 0.5-Labelllumäquivalenten des Extrakts von Ophys-Blüten. Aus einem größeren Versuch sind für 3 Bienenarten und 3 Ophys-Arten je 1 Antennenpräparat bzw. je 1 Reizquelle herausgegriffen. Der Balken markiert die Reizsekunde; Amplitudeneichung (rechts oben) 0.8 mV. Ophys lutea löst an Andrena pubescens, die Ophys-Arten sphecodes ssp. sphecodes und arachnitisformis an A. jaciobi, und die Arten bombyliflora und apifera an Eucera tuberculata hohe bis sehr hohe EAG-Antworten aus. Bei derselben Reizanordnung ist O. bombyliflora oder O. apifera für A. pubescens völlig unreaktiv, ebenso O. lutea für E. tuberculata; in der weiteren Kombinationen werden schwache Antworten registriert. In dieser Weise wurde auf 3 Konzentrationsstufen die Wirkung des Labellenextrakts von 12 Ophys-Formen auf Männchen von 30 Bienarten bestimmt.


Stellt man dieses im EAG-Test erhaltene Bild den Ergebnissen der langjährigen Freilandversuche Kullenbergs gegenüber, so ergeben sich einerseits sehr gute Übereinstimmungen, in einigen Punkten jedoch auch Abweichungen:


Littlea. Aufgrund ihrer spezifischen Wirkung auf bestimmte Andrena-Arten grenzt bereits Kullenberg (1961) diese Ophrys-Art als eigenen Dufttyp ab. Dies wird jetzt voll bestätigt durch die elektrophysiologische Prüfung des Labellenextrakts, der an Δ3 von Andrena pubescens Ol. sehr hohe und spezifische Antworten auslöst (Abb. 1, 2). Für die weiteren Andrena-Arten erwiesen sich die littlea-Präparate dagegen stets als nur schwach wirksam bis unwirksam.

Fusca. Keine der geprüften Bienenarten zeigte starke EAG-Antworten auf Labellenextrakte von O. fusca. Das von mehreren Standorten Südfrank-


In den meisten der chemisch analysierten Ophrys-Arten wurden zyklische Sesquiterpenkohlenwasserstoffe der Summenformel C_{15}H_{24} festgestellt, von denen die 3 häufigsten in den Massenspektren und GC-Retentionszeiten mit Longicyclen, γ-Cadinen und α-Copaen übereinstimmten (G. Bergström, persönl. Mitt.). Bei einigen Ophrys-Arten traten noch nicht näher bestimmte Sesquiterpenalkohole und Diterpene hinzu. Es war naheliegend, die 3 genannten sowie verschiedene nächstverwandte Sesquiterpene als reine Stoffe elektrophysiologisch auf ihre Wirksamkeit zu prüfen. Am Beispiel der Langhornbiene Eucera tuberculata soll hier gezeigt werden, daß ein solches Verfahren tatsächlich zu weiteren Aufschlüssen führt. Geprüft wurden an männlichen Antennenpräparaten dieser Art:

a) Longicyclen, Longifolen, Longipinen (tetrazyklisch);
b) α-Copaen, β-Copaen, α-Ylangen, β-Ylangen (tricyklisch);
c) α-Amorphene, β-Cadinen, γ-Cadinen, δ-Cadinen, ε-Cadinen, ε-Bulgaren, γ-Muurolen, ε-Muurolen (bicyklisch mit Cadinen-Gerüst);
d) zyklische Sesquiterpenkohlenwasserstoffe mit abweichendem Kohlenstoffgerüst wie α-Guaien, β-Guaien, α-Gurjunen, α-Humulen, α-Cedren, β-Cedren, β-Curcumene, α-Curcumene, Caryophyllen, Calaren; sowie
e) eine Auswahl von Sesquiterpenalkoholen.

Die unter a, b und d genannten Substanzen erwiesen sich bis zu der sehr hohen Reizquellenmenge von 1 mg als unwirksam oder nur sehr schwach wirksam. Dasselbe gilt für die geprüften Alkohole.

Abb. 3. Eucera tuberculata δ. Abgestufte EAG-Antworten (Symbols wie in Abb. 2) auf 12 Sesquiterpenkohlenwasserstoffe. Bei der Reizquellenbeladung 100 μg werden auf γ-Cadinen sehr hohe, auf die weiteren Testsubstanzen dagegen keine oder nur sehr schwache Antworten registriert.


Labellenextrakten bestimmter Ophrys-Arten gehen
dagegen von Extrakten von Eucera aus. Bisher
ist keine Komponente eines weiblichen Sexual-
pheromons einer Eucera-Art chemisch bekannt;
es kann somit noch nicht gesagt werden, ob be-
stimmte Ophrys-Inhaltsstoffe mit dem weiblichen
Pheromon einer Eucera-Art identisch sind oder
nur dessen Wirkung imitieren. Alle bisher durchge-
führten EAG-Messungen wären mit der Annahme,
dab Eucera-γ-Cadinen produzieren, vereinbar.
Für eine breite intarspezifische Wirks-
samkeit der weiblichen Sexualpheromone der Eucera-
Arten spricht der Befund, daß γ-Extrakte auch
taxonomisch entfernter stehender Arten wie E.
caspica an δ-Extrakten E. tuberculata hohe EAG-
Antworten auslösen.

Ungleich differenzierter ist demgegenüber das
Bild in der zweiten näher untersuchten Gattung,
Andrena. Von den 24 geprüften Arten zeigten
nur die Männer von A. pubescens und A. jacobi sehr
starke EAG-Antworten auf Labellenextrakte be-
stimmter Ophrys-Arten (Abb. 2). Die übrigen Arten
reagierten nicht oder nur relativ schwach auf die
Ophrys-Reizquellen, dagegen in den untersuchten
Fällen z.B. stark auf Extrakte art eigener γ. Dies
zeigt, daß nur ein Teil der für Andrena-δ-Extrak-
te wirksamen Attractivstoffe in den Ophrys-
Blüten vertreten ist. Über die mögliche Vielfalt sol-
cher Andrena-locksstoffe können beim gegenwärtigen
Bilden noch kaum Aussagen gemacht werden:
Die 24 näher untersuchten Arten repräsentieren
nur 2% der 1,200 beschriebenen Arten dieser
riesigen Gattung, und nur 13 der 51 von Warncke
(1968) für die westliche Paläarktis abgegrenzten
Untergattungen. Selbst auf nächstverwandte Arten
lassen sich die erhaltenen Ergebnisse noch kaum
übertragen. Die einzige Ausnahme in dieser Hinsicht
bietet die bereits erwähnte, zu A. jacobi sehr nahe
verwandte A. trimerera K., für die aufgrund ihres
Freilandverhaltens (spezifische δ-Extraktionen an
O. sphecodes sphecodes und an O. arachnitiformis,
s.o) ein ähnliches EAG-Reaktionsspektrum wie
für A. jacobi erwartet werden kann. Ob dieser
Reaktionstyp dagegen noch bei weiteren Arten
derselben Untergattung Hoplandrena auftritt (z.B.
bei rosae Pz. oder bei ferox Sm.), bleibt zu prüfen.
Die sehr hohe EAG-Reaktion auf O. lutea ist
beim gegenwärtigen Stand auf A. pubescens be-
schränkt und zeigt sich schon nicht mehr bei der
zur selben Untergattung Melandrena gestellten A.
nigroaenea (Abb. 2); von Interesse wären Unter-
suchungen an zu pubescens besonders nahe ver-
wendten Arten wie limata Sm. oder thoracica F.
Gerade lutea produziert unter allen bisher chemisch
analysierten Ophrys-Arten das reichste Spektrum
an Sesquiterpenen (G. Bergström, persönl. Mitl.)
und damit möglicherweise mehrere für Andrena-δ-
attractive Substanzen. Als wichtige im EAG-Test
noch nicht geprüfte Untergattung u.a. Chloran-
drena zu nennen, deren Arten humilis Im., cinerea
Brulé, taraxaci Gir., senecionis Pér. und nigro-
olivacea Dours wiederholt als spezifische Pollinatoren
von O. lutea beobachtet wurden (Kullenberg, 1961,
1973a). Mit Einbeziehung weiterer Andrena-Arten-
gruppen könnten aber möglicherweise auch Arten
gefunden werden, die auf die bisher so wenig
wirksamen fusca-Präparate ähnlich hohe EAG-
Reaktionen zeigen wie A. pubescens auf O. lutea.

Substanzen wie γ-Cadinen, α-Copaen und Longi-
cyclen stehen wahrscheinlich in engem biosynthe-
sischem Zusammenhang. Auch unter den noch
unbekannten weiteren Ophrys-Attraktivstoffen kön-
nen Moleküle dieses Bautyps erwartet werden.
In diesem Stadium der Untersuchung erscheint es
aussichtsreich, zahlreiche weitere Abwandlungen
derer bereits bekannten Moleküle vergleichend auf
spezifische EAG-Wirkungen zu testen. Analog
der Verfahren haben bereits in mehreren anderen Insek-
tengruppen die Aufdeckung bis dahin unbekannter
Pheromonstrukturen ermöglicht (Beispiele s. Pries-
nier, 1972). Für die hier interessierende Gruppe von
Sesquiterpenen stehen einem solchen Verfahren
die durch die Materialbeschaffung her noch
große Schwierigkeiten entgegen, sind doch erst für
einen kleinen Teil der theoretisch möglichen Iso-
eren Synthesen oder natürliche Vorkommen
beschrieben. Neben reinen Substanzen können für
Testzwecke jedoch auch Fraktionen bestimmter an
Sesquiterpenen reicher ärzlicher Öle herange-
zogen werden. So löste eine „Tallöl-Fraktion 66--77“
(G. Bergström) δ-Extrakt A. jacobi sehr hohe
EAG-Antworten aus, während sie für alle weiteren
geprüften Arten nur wenig wirksam war. Die Frak-
tion könnte damit möglicherweise den sphecodes/
araunenformis-Attraktivstoff enthalten, dessen An-
reichung und Isolierung gegenwärtig versucht
wird. Wichtige Hinweise ergeben sich auch aus dem
Verhalten von Hypermopteren-δ-Extrakt gegenüber reinen
Substanzen, Substanzmischungen, Fraktionen usw.
im Feldtest (Kullenberg, 1961, 1973b). Die bisherigen
Erfolge lassen hoffen, daß in enger weiterer Zusam-
nenarbeit von Chemikern, Feldbiologen und Sinnesphysiologen die Attraktivstoffe einer Anzahl von Ophrys-Arten in nächster Zeit chemisch und in ihrer Wirkungsweise aufgeklärt werden können.

**SCHLUSSBEMERKUNG**

Die spezifische Attraktion der Männchen bestimmter Insektenarten an Orchideenblüten, mit anschließender Pseudokopulation, ist nicht auf die Gattung Ophrys beschränkt. Analoges Verhalten wurde beschrieben für Schlupfwespen-3,3'-der Gattung Lissopinpla gegenüber Cryptostylis-Arten (Coleman, 1927-38); für Raupenfliegen-3,3'-Tachinidae gegenüber Telepogon-, Trichoceros- und Stellitabium-Arten (Dodson, 1962); für 3,3'-stachellose Bienen der Gattung Trigona gegenüber Trigonidium (Kerr u. Lopez, 1963); und dürfte sich mit näherer Beobachtung noch in weiteren Orchideen-Gruppen nachweisen lassen (vgl. van der Pijl u. Dodson, 1966). Für keine dieser Orchideen-Gattungen wurde die chemische Analyse von Blüteninhaltstoffen in Angriff genommen.


**ZUSAMMENFASSUNG**

Die spezifischen Attraktivwirkungen von Blütenduftstoffen bestimmter Ophrys-Arten (Orchidaceae) auf Männchen bestimmter Arten, oder Artengruppen, aku-


LITERATUR


—1928. Pollination of an Australian orchid by the male ichneumonid Lissopimpla semipunctata Kirby. Trans. R. Entomol. Soc. Lond. 76, 533–537.


einer Monographie und Ikonographie der Gattung *Ophrys*. Chernex-Montreux.


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Tafel 1

1. *Ophrys arachnitiformis.*
4. *Ophrys lutea.*
5. *Ophrys insectifera.*
Aufnahmen 1–8, Verfasser; 9, B. Kullenberg.
Geranium and Neral as Main Components in Cephalic Secretions of Four Species of Prosopis (Hym., Apidae)  

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Ecological Station of Uppsala University, Färjestaden, Sweden  

ABSTRACT  
Bergström, G. and Tengö, J. (Ecological Station of Uppsala University, Färjestaden, Sweden.) Geranium and neral as main components in cephalic secretions of four species of Prosopis (Hym., Apidae).  
ZOOIM, Suppl. 1: 55-59, 1973  
Volatile, cephalic secretions from four species of the genus Prosopis F. (Hym., Apidae), viz. Prosopis communis Nyl., Prosopis confusa Nyl., Prosopis kyalinata Sm. and Prosopis pectoralis Först. have been analysed with the help of gas chromatography and mass spectrometry. We have found that the secretions from both sexes contain the two isomeric acyclic monoterpane aldehydes geraniol (3,7-dimethyl-2-trans,6-octadiene-1-al) and neral (3,7-dimethyl-2-cis,6-octadiene-1-al) as main components together with other isoprenoids and straight chain hydrocarbons.  

INTRODUCTION  
This investigation was initiated by earlier work on the importance of chemical stimuli for the sexual attraction and pollination behaviour of aculeate Hymenopteran insects (Kullenberg, 1956, 1961). One finds in the literature several early observations of the occurrence of odours from solitary bees, e.g. concerning the genera Andrena, Melitta and Nomada, referred to by Perkins (1919). In papers by Dufour (1841), Bordas (1895), Heselhaus (1922) and Jacobs (1925) the morphology and histology of various glands of solitary bees are described. A recent review of mandibular gland systems of certain bees is that of Nedel (1960), who observes that the mandibular gland is well developed in Prosopis.  
Prospis is considered as a primitive group among apids, sometimes classified as a special family Colletidae (Mehely, 1935; Michener, 1944; Sustera, 1958). It is a worldwide-spread genus with a large number of species.  

MATERIALS AND METHODS  
Data concerning the collection and analyses of the bees are given in Table 1. The animals were collected in Ölands Skogsby and Skarpa Alby on the island of Öland, southern Sweden, and analysed immediately in most cases. Some of the heads were kept in hexane at low temperature (~20°C) until utilized.  
The analysis has been performed in Ölands Skogsby with a LKB 9 000 gas chromatograph-mass spectrometer and with a Perkin-Elmer 900 gas chromatograph modified for use with a split-free intake system (Ställberg-Stenhagen, 1972). For the combination instrument a 2 m long column with 2% Silicone SE-30 “Ultraphase” on dimethyl dichlorosilane treated Chromosorb G 80-100 mesh was used. The material was introduced into the gas chromatograph through a precolumn (Bergström, 1973). The separate gas chromatograph was equipped with a glass capillary column 23 m long (number 66). This column was coated with silicone OV-101 and measured 88 000 theoretical plates for methyl n-pentadecanoate.  
Heads, or in a few cases hexane extracts of heads, were introduced in either of the gas chromatographs and the volatile material driven off to the first part of the analytical column by programmed heating to +200°C for 10 min. The temperature of the column oven was programmed 4°C per minute.  
Taxonomical determination according to Elfving (1951).  

RESULTS  
A typical capillary gas chromatogram obtained from one head of a male Prosopis confusa is shown in Fig. 1. Reference hydrocarbons were added to the head of another individual of this species. The cor-
Table I. Materials of Prosopis (heads)

<table>
<thead>
<tr>
<th>Species</th>
<th>Sex</th>
<th>n</th>
<th>Date of collection</th>
<th>Date of analysis</th>
<th>Analytical method</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. communis</em></td>
<td>female</td>
<td>5</td>
<td>5.8.70</td>
<td>5.8.70</td>
<td>GCMS 70-58</td>
</tr>
<tr>
<td></td>
<td>female</td>
<td>1</td>
<td>22.7.71</td>
<td>10.8.71</td>
<td>CGC 72*</td>
</tr>
<tr>
<td></td>
<td>male</td>
<td>1</td>
<td>25.7.71</td>
<td>25.7.71</td>
<td>GCMS 71-67</td>
</tr>
<tr>
<td></td>
<td>female</td>
<td>1</td>
<td>17.7.72</td>
<td>17.7.72</td>
<td>CGC 469</td>
</tr>
<tr>
<td></td>
<td>female</td>
<td>1</td>
<td>19.7.72</td>
<td>20.7.72</td>
<td>CGC 469</td>
</tr>
<tr>
<td><em>P. confusa</em></td>
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<td>1</td>
<td>16.7.71</td>
<td>16.7.71</td>
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<tr>
<td></td>
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<td>21.7.71</td>
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<td></td>
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<td>24.7.71</td>
<td>10.8.71</td>
<td>CGC 73*</td>
</tr>
<tr>
<td></td>
<td>male</td>
<td>1</td>
<td>24.7.71</td>
<td>25.8.71</td>
<td>GCMS 71-112*</td>
</tr>
<tr>
<td></td>
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<td>1</td>
<td>25.7.71</td>
<td>26.7.71</td>
<td>GCMS 71-68</td>
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<td>1</td>
<td>11.6.72</td>
<td>12.6.72</td>
<td>CGC 380</td>
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<tr>
<td></td>
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<td>11.6.72</td>
<td>12.6.72</td>
<td>CGC 381</td>
</tr>
<tr>
<td></td>
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<td>1</td>
<td>19.7.72</td>
<td>20.7.72</td>
<td>CGC 468</td>
</tr>
<tr>
<td><em>P. hyalinata</em></td>
<td>female</td>
<td>3</td>
<td>20.7.71</td>
<td>20.7.71</td>
<td>GCMS 71-63</td>
</tr>
<tr>
<td></td>
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<td>25.7.72</td>
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<td>CGC 486</td>
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<tr>
<td><em>P. pectoralis</em></td>
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<td>1</td>
<td>6.8.71</td>
<td>6.8.71</td>
<td>GCMS 71-86</td>
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<td>1</td>
<td>6.8.71</td>
<td>7.8.71</td>
<td>GCMS 71-88</td>
</tr>
</tbody>
</table>

GCMS = Gas chromatography/mass spectrometry.
CGC = Capillary gas chromatography
* Analyses so marked were made with an extract, in hexane. In all other analyses, whole heads, together with the excised mandibles, were put directly in the precolumn tube or in the splitter-free intake system.

The corresponding capillary gas chromatogram is given in Fig. 2.

The main components of the secretion have been identified as the two isomers of citral, geranial (3,7-dimethyl-2-trans,6-octadiene-1-al) and neral (2,7-dimethyl 2-cis,6-octadiene-1-al). Of these, geranial is the largest component. Mass spectra of the natural compounds are given in Figs. 3 and 4. (Reference samples of citral were obtained from Firmenich & Cie., Geneva, Switzerland.) The mass spectra of the two isomers, although generally similar, show some characteristic differences, particularly in the high mass end. Thus the relative proportions between the fragments M, M-15,
M-18, M-29, M-33 and M-43 distinguish the two isomers clearly. Tricosane and pentacosane were also identified by their mass spectra.

A few other isoprenoid compounds, probably related biosynthetically to citral, were found to be present in the secretions. Their structure has so far not been determined. As shown in Figs. 1 and 2, a minor component, not fully resolved from the next largest component (neral), occurs in the chromatogram. This compound is identical with neral as shown by its mass spectrum and its retention index.

Capillary gas chromatograms of heads from males and females of the other species studied all show the same general composition. As examples, chromatograms of Prosopis hyalinata male and Prosopis communis female are given in Figs. 5 and 6, respectively. These chromatograms reveal the typical proportions between the two citral isomers.

**DISCUSSION**

Citral has earlier been found in the mandibular gland secretion of ants of the genera *Atta* (Butenandt et al., 1959) and *Acanthonylops* (Chadha et al., 1962), stingless bees of the genus *Trigona* (Blum et al., 1970) and in robber bees of the genus *Lestrimelitta* (Blum, 1966). Citral is also produced by the Nasonoff gland of the honeybee (Shearer & Boch, 1966; Butler & Calam, 1969; Weaver et al., 1964).

In *Trigona subterranea*, citral functions as a trail substance between the nest and the food find whereas its role for *Lestrimelitta limao* is to cause chaotic behaviour in small bees of the genera *Melipona* and *Trigona*, the nests of which are robbed by *Lestrimelitta*. In the ant species *Atta sexdens rubropilosa* and *Acanthonylops claviger* citral functions as an alarm pheromone. The honeybee makes use of citral and other components of the
Nassanoff gland secretion in connection with foraging behaviour.

We have found that citral occurs in several species of Prosopis and in both sexes. According to Kullenberg (1956) it might be a common phenomenon in solitary bees that both sexes possess the same odour.

Citrál was also identified by us in the androconial secretion of Pieris napi (Bergström & Lundgren, 1973). This finding together with the identification of citral in Prosopis are further examples of the widespread occurrence of citral in behaviour-releasing secretions of insects.

ACKNOWLEDGEMENTS

We wish to extend our sincere acknowledgements to the Swedish Natural Science Research Council, the Axel and Margaret Ax:son Johnson Foundation and the Ekhaga Foundation for financial support and to Professor Bertil Kullenberg, Professor Stina Stalberg-Stenhagen and Professor Einar Stenhagen for encouragement and helpful suggestions.

REFERENCES

Bergström, G. 1973. Studies on natural odoriferous compounds. VI. Use of a pre-column tube for the quanti
Geraniol and nerol in Prosopis cephali secretion

Fig. 7. Structures of geraniol (a) and nerol (b).

- Ställberg-Stenhagen, S. 1972. Studies on natural odoriferous compounds. V. Splitter-free all glass intake system for glass capillary gas chromatography of volatile compounds from biological material. Chemica Scripta 2, 97-100.


2,3-Dihydro-6, trans-farnesol: Main Component in the Cephalic Marker Secretion of Bombus jonellus K. (Hym., Apidae) Males

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ABSTRACT


We have analysed the cephalic marking secretion of males of the bumble-bees species Bombus jonellus. The main component was identified as 2,3-dihydro-6, trans-farnesol. 2,3-Dihydro-6,trans-farnesol and tricosane were found to be present in the secretion in appreciable amounts.

The analysis was brought about by capillary gas chromatography, employing a splitter-free intake system and combined gas chromatography-mass spectrometry.

INTRODUCTION

This investigation forms part of a larger study of the marking secretions of male bumble-bees. A few reports of results from other species have already been published (for references, see below). The present work is a continuation, in particular, of the studies of species, which are to be found more abundantly or exclusively in the northern part of Scandinavia. An account of results from the analysis of two forms of Bombus lapponicus F. has already been reported (Bergström & Svensson, 1973). Bombus jonellus K., now studied, belongs to the same subgenus as Bombus lapponicus, viz. Pyrobombus D.T.

MATERIAL

The bees were collected in 1972 at Abisko, Torne Lappmark, Sweden, in birch-wood while feeding (see Table I). The techniques of preparation in the

1 This report forms part XVII of the series “Studies on natural odoriferous compounds”.

field and in the laboratory have been described earlier (Bergström & Svensson, 1973). From extracts of heads, an odour reminiscent of farnesol and aliphatic acetates has been noted. The taxonomical determinations have been made according to Elfving (1960). Some collection and analysis data are summarized in Table I.

ANALYTICAL METHODS

The analytical methods employed in this analysis are the same as those described in recent publications (Bergström et al., 1973; Stållberg-Stenhagen, 1972; Bergström, 1973). Capillary gas chromatography, run with the use of a splitter-free all glass intake system, and combined gas chromatography-mass spectrometry have been employed in the analysis. In both techniques temperature-programmed operation was used.

The retention indices of the natural components on the capillary column were compared with those of reference hydrocarbons and with those of other reference compounds available in our laboratory.

RESULTS

Fig. 1 shows a gas chromatogram of a portion of an extract, in hexane, of 3 male heads (collection number 37) of B. jonellus. This chromatogram was obtained from gas chromatography in conjunction with mass spectrometry. The temperature of the gas chromatographic oven was programmed from +40°C to +220°C with 4°C/min. Mass spectral registrations are indicated by arrows.

In Fig. 2 a strong capillary gas chromatogram (No. 679) is shown with reference hydrocarbons
Table I. Data regarding collection and analysis of Bombus jonellus

<table>
<thead>
<tr>
<th>Collection no.</th>
<th>Date of collection</th>
<th>No. of heads</th>
<th>Type of analysis</th>
<th>No. of analysis</th>
<th>Collected on</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>12 July 1972</td>
<td>1</td>
<td>CGC</td>
<td>679</td>
<td>Astragalus alpinus</td>
</tr>
<tr>
<td>31</td>
<td>19 July 1972</td>
<td>1</td>
<td>CGC, GC/MS</td>
<td>630, 634, 638</td>
<td>Chamaenerion angustifolium</td>
</tr>
<tr>
<td>37</td>
<td>22 July 1972</td>
<td>3</td>
<td>CGC/MS</td>
<td>552, 26.9.72</td>
<td>Chamaenerion angustifolium</td>
</tr>
</tbody>
</table>

CGC = Capillary Gas Chromatography.
GC/MS = Combined Gas Chromatography and Mass Spectrometry.

In the first mentioned report (Bergström et al., 1968) 5 of the 8 possible mono-unsaturated farnesol isomers were synthesized by means of partial hydrogenation of 2-t,6-t and 2-c,6-t farnesol, employing hydrazine. These compounds produce characteristic mass spectral fragmentation patterns. Comparison between these (see Bergström et al., 1968, pp. 459-460) and the mass spectrum in Fig. 3 indicates that this compound is a 2,3-dihydrofarnesol just as in the case of B. terrestris. Capillary gas chromatographic retention indices are identical, 1672, for component 2 of the secretion from B. jonellus and for synthetic 2,3-dihydro-6,trans-farnesol.

Furthermore, a mixture of the secretion from B. jonellus and synthetic 2,3-dihydro-6,trans-farnesol, in equal amounts, showed a harmonious peak with retention value 1672 (see Fig. 4). The absolute configuration of component 2 of B. jonellus has so far not been determined.

In an earlier publication (Bergström et al., 1968) the main component in the cephalic marking secretion of male B. terrestris L. was identified as 2,3-dihydrofarnesol. The absolute configuration of this compound, called terrestrol, was later established (Ställberg-Stenhagen, 1970) and the structure was proved to be (-)-3L,7L,11-trimethyl-dodeca-6,10-dien-1-ol. The two enantiomers of 2,3-dihydro-6,trans-farnesol were also synthesized (Ahquist & Ställberg-Stenhagen, 1971).

![Fig. 1. Gas chromatogram of a portion of an extract of the cephalic secretion, in hexane, of B. jonellus, male.](image-url)
Component 1 was identified by its mass spectrum and retention index, 1595, as 2,3-dihydro-6,trans-farnesal. This compound was earlier found to be present in a cephalic secretion from worker ants of the species Lasius flavus F. (Bergström & Löfqvist, 1970). The mass spectral fragmentation pattern is particularly characteristic in the higher mass end of the spectrum, including a characteristic peak at $m/e = 179$, probably due to loss of the ion [CH$_2$CHO$^+$. Tricosane, component 3, was also identified by mass spectrum and retention index.

Fig. 2 shows clearly the dominance in the secretion of component 2. It also shows that there are some components present in amounts comparable to those of 2,3-dihydro-6,trans-farnesal and tricosane. They have so far not been identified.

For comparison, another extract of the cephalic secretion from one head of a male *B. jonellus* was analysed by capillary gas chromatography. This individual (collection No. 31) showed the same general relative composition as the one referred to above (collection No. 37).
DISCUSSION

2,3-Dihydro-6,trans-farnesol represents the main component of the secretion of *B. jonellus*. In this respect it is similar to the secretion of *B. terrestris*, reported earlier (Stållberg-Stenhagen, 1972). On the other hand, there are characteristic differences between the two species. *B. jonellus* possesses a considerable amount of 2,3-dihydro-6,trans-farnesol, whereas *B. terrestris* in addition to terrestrol has appreciable amounts of ethyl dodecanoate. The two species resemble each other somewhat in the coloring of the body, but exhibit clearly different morphology of the genital apparatus.

A comparison between the composition of the marking secretions of *B. jonellus* and some other bumble-bee species stresses the similarities to *B. terrestris*. In a preliminary report (Kullenberg et al., 1970) 13 species of the genus *Bombus* and 6 of the genus *Pathyurus* were compared. Dihydrofarnesol was only found to be present in the secretion of *B. terrestris*.

ACKNOWLEDGEMENTS

Most of this work was performed at the Ecological station of Uppsala University, Ölands Skogsby, and at the Field station of the Royal Swedish Academy of Sciences in Abisko. We thank the heads of these stations, Professor Bertil Kullenberg and Professor Gustav Sandberg, respectively, for providing working facilities. We also thank Professor Stina Stållberg-Stenhagen for providing a sample of 2,3-dihydro-6,trans-farnesol.

Financial support from the Axel and Margaret Ax:son Johnson Foundation and the Swedish Natural Science Research Council is gratefully acknowledged.

REFERENCES


Stålborg-Stenhagen, S. 1972. Studies on natural odoriferous compounds. V. Splitter-free all glass intake system for glass capillary gas chromatography of volatile compounds from biological material. Chemica Scripta 2, 97–100.
Androconial Secretion of Three Species of Butterflies of the Genus Pieris (Lep., Pieridae)

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ABSTRACT

Bergström, G. and Lundgren, L. (Ecological Station of Uppsala University, Färjestaden and Laboratory of Ecological Chemistry, University of Göteborg, Mölndal, Sweden.) Androconial secretion of three species of butterflies of the genus Pieris (Lep., Pieridae).

ZOO, Suppl. 1: 67-75, 1973

Chemical analysis, performed with the help of combined gas chromatography/mass spectrometry as well as capillary gas chromatography, gave the following principal results. (1) The androconial secretions of the three species of Pieris studied were composed of several components. (2) The chemical composition was quite different in the three species. (3) It is possible to obtain analytical results from single individuals, which makes it feasible to compare them. (4) The main components of the androconial secretions of P. napi (L.) were identified as the two isomers of citral, geranial and neral, in the approximate proportions 1:2. What is known about the fine structure of the Pieris scale has been surveyed. The androconial scales of P. napi (L.) and P. brassicae (L.) have been photographed under a scanning microscope. Possible biological functions of the male scent are discussed.

INTRODUCTION

According to Aurivillius (1880) the specialized scent scales of male butterflies now known as androconial scales were first described by Baillif (1825) in Pieris rapae (L.). Deshamps (1835) found that these scales existed only in the males. The existence of scent scales in over 200 pierid species was proved by Watson (1865). Müller (1877) was the first to connect the androconial scale patches with the odour of male butterflies, perceivable for our nose. In the same year Scudder suggested the name androconia. It was early discovered (Watson, 1865) that the shape of the male scales was typical of the species. Nobody made systematic use of the taxonomic possibilities until Warren (1961, 1963a, b) started to examine the androconial scales of Pieris and their bearing on the question of speciation in the genus.

When new sensitive techniques of analysis made it possible to investigate the chemistry of odour signals, most work on Lepidoptera focused on the fascinating long-distance operating sex attractants of female moths. Very little has been done about butterfly scents in spite of the fact that most of the few investigations of the mating behaviour of butterflies indicate that also these "eye animals" are guided by mutual scent signals in the last phases of the courtship (lit. in Brower et al., 1965; Myers, 1972; Lundgren "A Comparing Analysis of the Courtship Behaviour in Some Species of Blues", to be publ.).

This paper is the first report from our work on odoriferous compounds secreted from the androconial scales. The common occurrence of scent-producing organs in Lepidoptera indicates that the evolution of chemical signals has implied considerable selective advantages. As Pieris napi is the first species where the androconial scent has been identified, we still do not know if the odoriferous compounds which are presented to the female in the last phases of the courtship, are species-specific aphrodisiac key signals. In the field of male pheromones in Lepidoptera, moreover, some papers have been published on the secretions of abdominal "hair-pencil" of danaid butterflies (lit. in Seibt et al., 1972). A few investigations of the pheromones from the abdominal brushes of male noctuid moths have also been made (Aplin & Birch, 1968, 1970; Birch, 1970; Grant, 1970). As far as we know, no female abdominal pheromone has been identified in any species of butterflies (Rhopalocera).
Table 1. Data regarding collection and chemical analysis of androconial secretion from Pieris napi, Pieris rapae and Pieris brassicae

<table>
<thead>
<tr>
<th>Species</th>
<th>Analytical method</th>
<th>Number of analysis with number of animals in parentheses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CGC</td>
<td>475 (2), 480 (1), 482 (1), 483 (1), 490 (1), 542 (1)</td>
</tr>
<tr>
<td>P. rapae</td>
<td>GCMS</td>
<td>70–103 (17), 71–16 (8), 71–29 (7)</td>
</tr>
<tr>
<td></td>
<td>CGC</td>
<td>484</td>
</tr>
<tr>
<td>P. brassicae</td>
<td>GCMS</td>
<td>70–104 (6), 71–30 (5)</td>
</tr>
<tr>
<td></td>
<td>CGC</td>
<td>547 (2), 635 (1), 636 (1)</td>
</tr>
</tbody>
</table>

GCMS = Gas chromatography/mass spectrometry.
CGC = Capillary gas chromatography.
All material was collected in the neighbourhood of the Ecological Station, Ölands Skogshy.

MATERIALS AND METHODS

In Table I, some data concerning collection and subsequent chemical analysis are given. As shown in the table, butterflies have been collected in 1970, 1971 and 1972, all in the neighbourhood of the Ecological Station, Ölands Skogshy, and analysed there. The material of P. brassicae (L.) analysed in Göteborg in 1973, was obtained as eggs from a laboratory stock held for twenty years by Dr B.O.C. Gardiner, Cambridge University, England (David & Gardiner, 1952).

Directly after collection, wings were cut off and put either on a precolumn tube of the gas chromatograph coupled to the mass spectrometer or transferred to the splitter-free system of one of the two capillary gas chromatographs used. The inlet systems of the gas chromatograph/mass spectrometer as well as the capillary gas chromatographs have been described earlier (Bergström, 1973; Ställberg-Stenhagen, 1972).

The wings from dead and dried specimens were used for the SEM-photographs in this paper. The wing surface was covered with evaporated coal, silver and gold. In some preparations (Figs. 9 and 10) the scales were extracted by gently pressing the wings against a stub covered with tape glue. The acceleration voltage was varied between 3–5 kV.

Type of microscope: Cambridge Stereo-Scan S-4.

RESULTS OF CHEMICAL ANALYSIS

P. napi

From 6 analyses of volatile compounds from wings of male P. napi using combined gas chromatography/mass spectrometry we have found that the dominating components present in the androconial secretion are the two isomers of citral namely, geraniol = trans-citral (3,7-dimethyl-2-trans,6-octadiene-1-al) and nerol = cis-citral (3,7-dimethyl-2-cis,6-octadiene-1-al). These components are present in quite large amounts, about 1 mg per individual, in the approximate proportions 1:2. Figs. 1 and 2 show capillary gas chromatograms (No. 483 and 490), with and without reference hydrocarbons, of the volatile compounds from wings of P. napi males.

![Fig. 1. Capillary gas chromatography of wings from one male P. napi with reference hydrocarbons added.](image-url)
Mass spectra of the dominating compounds are given in Figs. 3 and 4. By comparison with mass spectra and capillary gas chromatographic retention indices of citral, containing trans/cis isomers in proportions 1:2, obtained from Firmenich et Cie, Geneva, Switzerland, the identity could be established. The isomers of the reference were separated by preparative gas chromatography on a Hyprose S.P.-80 column. The mass spectrum of neral (Fig. 3) and the capillary gas chromatogram indicates that geraniol is also present in the secretion in somewhat minor amounts. Beside these components, several structural analogues to citral, with 8 and 9 carbon atoms, are present in the secretion in minor amounts. Tricosane, pentacosane and some other aliphatic compounds, hitherto unidentified, have also been found as small components.

_P. rapae_ and _P. brassicae_

Preliminary analyses of the androconial secretion from these species involving gas chromatography and mass spectrometry (see Table I) show that they contain a few main components, characteristic for each species. Citral has not been found, even in
Fig. 5. Capillary gas chromatogram of wings from one male *P. brassicae* with reference hydrocarbons added.

minor amounts, in any of them. The major components are present in somewhat less amounts than citral in *P. napi*. Tricosane was found to be present in both species. Figs. 5 and 6 show capillary gas chromatograms of the volatile secretion from *P. brassicae*. The chromatograms (Nos. 635 and 636) emanate from wings of separate individuals, 6 days old, bred in our insectarium.

**COMPARISON BETWEEN THE THREE SPECIES**

The results reported above show that the three species studied possess an androconial secretion, which has a chemical composition characteristic of each species. This confirms the observations made regarding their odours.

Studies going on regarding their behaviour in courtship will now be made with synthetic compounds.

We are collaborating with Mr Michael Boppré (Max-Planck-Institut für Verhaltensphysiologie, Seevissen), who is investigating the effects of the scents with electrophysiological methods.

Citral has been found to be a widespread trigger of behaviour in insects. It has been identified in mandibular gland secretions from several ants, where it functions as an alarm or trail substance. In the

Fig. 6. Compare Fig. 5. This chromatogram was run at higher sensitivity.
Nassanoff organ of the honey-bee it occurs in conjunction with several other substances. It is the major compound responsible for odour communication in association with foraging behaviour in this insect. Recently, citral has been found (Bergström & Tengö, 1973) in the cephalic secretions of male and female Prosopis bees. In that report references are given to some other findings of citral in insects.

**BIOLOGY**

**Structure and function of the androconial scales**

Barth (1949) has thoroughly investigated the fine morphology of the scales in *Pieris brassicae* (L.) and *P. rapae* (L.). He has also suggested a plausible explanation of the function related to structure. As the works of this author, many of which are written in Portuguese, are too little referred to, it may be justified to give a short survey of his main results on the *Pieris* scales. That account is illustrated with microphotographs from a SEM-investigation of the androconial scales, which we have just started. The androconial scales of the genus *Pieris* are arranged in rows alternating with the ordinary scales. The scent scales are completely hidden by the longer type of the contour scales. Compared with other known types of androconial scales the *Pieris* scales are characterised by their specialised peduncle. It is broadly connected to the heart-like incised base of the scale. The tube immediately swings forward beneath the scale and then abruptly turns again in the opposite direction, ending in a bladder (Figs. 9 and 10). From the invaginated bottom of the vesicle an exocuticular duct leads, continued by a fine canal piercing the endocuticle. The passage of the secretion from the glandular cell situated between the wing lamellae is blocked by the sharp curves of the S-shaped canal. The bladder is inserted in a cuticular cylinder on the wing surface. The odoriferous secretion is stored in the insertion bladder. When the reservoir is extended, the expansive power presses the bladder out of the cylinder. The secretion-loaded balloon is separating the parallel contour scales. As soon as the secretion pressure becomes high enough, small quantities of the secretion are pressed into the scale lumen.

Thanks to this ballast, the androconial scales are thrown out away from the wing surface by the centrifugal power when the courting male is vigorously fluttering around the female. In this way the S-shaped duct straightens and the secretion evaporates freely through the perforated superior membrane and through the end pores of the fringe threads (Fig. 8) now projecting between the rows of contour scales (Fig. 7). Apparently these structures of the *Pieris* scale are a highly specialized device designed to protect the delicate scent scales and to secure a concentrated spread of the odour at the right moment.

Warren (1961) has compared the androconial scales of *Pieris napi*, *P. brassicae* and *P. rapae*. He considers the *brassicae* scale to be so specialized that he follows those who have excluded the species from the genus *Pieris*. It is too early to draw any conclusions based on the structure of the main components of the wing scents. The racial variation especially of *Pieris napi* is well known although it has suffered from excessive naming. We hope that it will be possible to follow the phylogeny of the isolating mechanisms on a molecular level.

*Pieris ergane* (Hbn.) which has been shown by Lorković (1953) to belong to the *napi* group, smells fainter but very much like the males of *P. napi*.

**Biological importance of the male scents**

As the behavioural and electrophysiological experiments with the *Pieris* scents are only initiated, a discussion of the biological importance of the androconial secretion must be fairly speculative. Conceivable biological functions of the male citral scent of *Pieris napi* can be tabulated as follows.

1. It may act as an arrestance of the escape flight of the female. This function of the male butterfly perfumes suggested by many authors seems highly probable, as the *napi* males during the aerial pursuit vigorously flutter around the female now and then striking the wings against her. It ought to be tested by releasing de-odourized males. This is comparatively easily done with the abdominal "hairpencils" of male Danaid butterflies. According to Pliske & Eisner (1969) odour-deprived males of the queen butterfly (Danais guippus berenice) are capable of bringing their mates to alight but fail to induce them to remain alighted and to copulate.

2. It may suppress the "mate-refusal posture" of the female. An unwilling *napi* female responds to the approaching male by abruptly opening the wings and lifting the abdomen upright. This behaviour is
found in all three species. Obara & Hidaka (1964) in cage experiments with *Pieris rapae crucivora* (Boisdouval) observed this behaviour only in mated females. By separating the pairs of *Limenitis camilla* at different times Lederer (1960) showed that a similar behaviour in this species is not provoked until the spermatophore is transferred.

Our own observations are in agreement with the results of Petersen & Tenow (1954) on *Pieris napi*.

Though the mate refusal behaviour is especially intense in new-mated females, it also occurs in insufficiently activated unpaired females. We have found the behaviour very frequent in our rearing of *Pieris brassicae* in artificial light. The prenuptial "fluttering around" the female which according to Barth (1949) is necessary to release the androconial scent, is in that case practically absent. Perhaps it is not released by the colour signals of the female wings, which are altered by the artificial light. These observations indicate that the androconial scent is a signal which suppresses the mate refusal posture.

3. It may have additional unknown aphrodisiac effects in the final phases of mating. This possibility seems to be ruled out by the successful handpairing of pierid butterflies, which indicates that the final phases of mating behaviour are guided by mutual tactile stimuli (Clarke, 1952; Lorković, 1953; Chovet, 1970).

4. It may be a species-specific signal actively preventing cross-breeding through its function as key signal for the behaviour already mentioned. Our nose and the chemical results so far tell us that the three investigated species of *Pieris* have different androconial scents. The sex pheromones are, in contradiction to the original definition, not necessarily
species-specific. In fact this is what is to be expected as only in some cases the evolution has followed the olfactory mode of building up the isolating mechanisms. However, two chemically identical scent substances of the male wings may act as species-specific signals on account of various auxiliary factors, e.g. the way of presenting the pheromone or by coeffects from other substances.

The androconial scents are scarcely the only stimuli that “fit” the odour-released phases of the female behaviour. Until we know at what extent odour deprived napi males copulate, or we have electrophysiological evidence of the stimulating effects, we cannot say anything about the relative releasing strength of the odour signals.

5. It may serve as a repellent on other males. Other males are often approached. A persistent courting of other males is disadvantageous. This speaks for a selection value of the repellent effect of the male scent besides the visual differences between the sexes. Obara & Hidaka (1964) noticed in Pieris rapae crucivora that a flying male, within a distance of about 30 cm, provoked a “flutter response” in a resting male. They first considered it to be a visual “I am a male”-signal. This hypothesis was abandoned as their further observations showed that the flutter response causes the flying male to approach the resting male purposefully. They could not find any adaptive significance of that signal effect.

We are of the opinion that the wing flutter of the male is highly adapted if it releases “anti-courting odours”. By comparison, the brief approach reactions of the flying males provoked by the wing flutter must have an insignificant negative selection value.

6. It may attract other males during the social swarm flight and when the males are chasing each other. Our observation of the aerial swarming flight in Pieris napi and P. brassicae are in agreement with the experiences of Obara (1970) with P. rapae. The behaviour is usually observed in such cases when a female fails to alight when pursued by the male. The pair attracts other males which in their turn increase the attractivity of the group until sometimes a swarm of males is surrounding the female. As shown by Obara the attraction of male wings is increased when wing movements which are a key-signal of the approach reaction, are added to the weak signal effect of male colour.

Obara (1970) writes “These responses are disadvantageous to males because they waste time and energy which would otherwise be concentrated on finding the female”. 
As we see it, the social flight is apparently provoked by the super-normal “wing movements effect” during the round fluttering aerial pursuit phase of the courtship. The intensity is enlarged for every male that joins the company. Concerning the biological meaning of the social swarm flight, a hypothesis by Temple (1949) merits discussion. She claims that sustained long-distance courtship flight occurs in species which are liable to sudden expansions in range. A common observation is that the whirling flight brings the pair to earth at a considerable distance from the starting point. Our impression is that the vertical and the high altitude horizontal element in the aerial phase of the courtship is wider in natural patchformed habitats than over great monocultural cabbage fields.

7. It may act as a predator deterrent. This possibility is suggested by Eisner (pers. com.). If the hypothesis is correct, it may be so that other sympatric white species may have mimetic advantages of their similarity to napi males.

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The authors wish to express their sincere thanks to Professor Bertil Kullenberg and Professor Einar Stenhagen for providing working facilities at the Ecological Station on Öland and at the Laboratory of Ecological Chemistry in Mölndal. The financial support of the Swedish Natural Science Research Council, the Axel and Margarete Axson Johnson Foundation and the Knut and Alice Wallenberg Foundation is gratefully acknowledged. We thank the Director of the Swedish Institute for Food Preservation Research, Professor Erik von Sydow for permitting us to use the scanning electron microscope. We are greatly indebted to Agr. Dr. Arne Liljemark and Mrs Gunnel Schallin for their skilled and interested work and for the microphotographs. As regards the English language the manuscript has been checked by Mr Erik Hedström to whom we express our gratitude. We are also very obliged to Mr Dag Lindqvist who wrote the abstract in French.

REFERENCES

Birch, M. G. 1965. Pre-courtship use of abdominal brushes by the nocturnal moth, Phlogophora meticulosa (L.) (Lepidoptera: Noctuidae). Anim. Behav. 18, 310-316.

ABRÉGÉ


L'analyse chimique fait à l'aide d'une combinaison de chromatographie à gaz et de masse spectrométrie ainsi qu'à l'aide de chromatographie à gaz capillaire donna les résultats principaux qui suivent. (1) Les sécrétions androconiales des trois espèces de Pieris étudiées étaient composées de parties différentes. (2) La composition chimique était tout à fait différente dans les trois espèces. (3) Il est possible d'obtenir des résultats analytiques des spécimens individuels, ce qui rend possible de comparer. (4) Les composants essentiels de la sécrétion androconiale des P. napi (L.) furent identifiés comme les deux isomères de citral: geraniol et nérale, dans les proportions 1:2.

Les écailles androconiales de P. napi (L.) et P. brassicae (L.) ont été photographiées sous un microscope scanning. Les fonctions biologiques possibles de l'odeur mâle sont discutées.

Nous avons fait un résumé de ce que l'on sait sur la structure fine de l'écaille du Pieris.


Scudder, S. H. 1877. Antigeny or sexual dimorphism in butterflies. Proc. Amer. Acad. of Art and Sciences VI, XII, 150.


Stålberg-Stenhagen, S. 1972. Studies on natural odoriferous compounds. V. Splitter-free all glass intake system for glass capillary gas chromatography of volatile compounds from biological material. Chemica Scripta 2, 97–100.

Temple, V. 1949. The courtship flight of butterflies as means of extending the range of certain species. Entomologist 82, 145–147.


Analytical Techniques in Pheromone Studies

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ABSTRACT

Ställberg-Stenhagen, S., Stenhagen, E. and Bergström, G.
(Institute of Medical Biochemistry, University of Göteborg,
Göteborg and Ecological Station of Uppsala University,
Färjestaden, Sweden.) Analytical Techniques in
Pheromone Studies.

A short review is given of the analytical techniques used
in pheromone studies. The necessity to improve the sen-
sitivity of the technique available is stressed and some
means of achieving this are briefly discussed.

The pheromones represent a rather heterogeneous
collection of chemical compounds which act as
chemical messengers between individuals of the
same (or closely related) species. Some compounds
of this type, e.g. many sex pheromones, exert their
biological action at extremely minute concentra-
tions in air (Butenandt & Hecker, 1961; Kasang, 1968;
Schneider, 1970). Their isolation from the insect
gland or from the air around the insect is con-
sequently difficult. In most works on insect pheromones
a large number of animals have been used. For
example in the classical work by Butenandt and
colleagues (1959) on the sex attractant of the silk
moth (Bombyx mori) 500 000 animals were used, and
the recent work on the gipsy moth (Parthenia
dispar) by Bierl et al. (1970) was carried out with
78 000 animals. There are several disadvantages in
using such large numbers of animals. It usually
requires breeding of the insects concerned and the
biology of many insects is not sufficiently known to
establish the proper breeding conditions. In the case
of the silk moth, Butenandt et al. had the advantage
that the insect was grown industrially in enormous
numbers for the production of silk. Suitable condi-
tions for breeding the insects were established even

1 This contribution forms pat. No. XVIII in the series
"Studies on natural odoriferous compounds".

in ancient times. In many other cases the breeding
conditions have to be established before breeding can
be successfully carried out. The artificial breeding
of many insect species is something of an art. The
intensity and spectrum of the light, the length of the
day and the food supply for larvae and imagines are
important factors, as are the detection and control
of predators, parasitic insects, and bacterial and
fungal diseases. Another matter is that in working
with large quantities of insects that give off volatile,
biologically very potent matter, part of the active
matter will evaporate and settle on the walls and
celing of the room, i.e. the laboratory will become
contaminated by highly active substances in such a
way as to make biological assays very difficult or
impossible to perform.

Furthermore, for taxonomic purposes it may be
very important to be able to work with single indi-
viduals. The new analytical techniques that have
become available now make this possible at least for
certain insects.

Few volatile pheromones have molecular weights
above 300 and most of them or suitable derivatives
can be investigated by gas chromatography. Glass
capillary columns of the Goly type give a high re-
olving power but have a low material throughput.
Furthermore, a splitter system is usually used in
order to make it possible to inject a sufficiently
small sample on the column, but this device causes
losses of up to 99% of the sample. A particularly
convenient intake system (cf. Figs. 1-3) has recently
been described by one of the authors (Ställberg-
Stenhagen, 1972). With this system the splitter
and the use of solvents have been eliminated.

One difficulty at present is that the on-line con-
ected detecting mass spectrometer is not sufficiently
sensitive to analyse the many small peaks in the
typical glass capillary gas chromatograms (cf. Fig. 4).
However, before discussing improvements in the
sensitivity of mass spectrometers and other analysing detectors, such as infrared spectrophotometers, we would like to discuss the problem of collecting and analysing volatile organic compounds, present in very small amounts in large volumes of free air. This situation exists, for example, in the neighbourhood of flowering plants and in the vicinity of still or moving insects. Air at the ambient temperatures usually encountered (0-35 °C) always contains relatively large amounts of moisture. At 20 °C the vapour pressure of water is 17.5 torr, which corresponds to 18.5 mg water per litre. If an attempt is now made to condense this water, the small amounts of the

Fig. 1. Details of an all-glass capillary intake system.

Fig. 2. Intake system and glass capillary column in position.

Fig. 3. Photograph of the intake system in position in a modified gas chromatograph Perkin-Elmer model 990. The white rod is a handle of Teflon.
active pheromone is condensed together with the water and later becomes adsorbed to the walls of the vessel. Many pheromones are unstable, i.e., polyunsaturated pheromone molecules may polymerize in the process, which may lead to large losses of biologically active substance. It occurred to us that it might be much better to use an ultracentrifuge for the enrichment of the heavier organic molecules in air. As is well known, the enrichment of large quantities of uranium 235 is successfully carried out by batteries of ultracentrifuges in spite of the small mass difference of 3 a.m.u. between $^{238}\text{U}$ and $^{234}\text{U}$. (The natural abundance of $^{235}\text{U}$ is 0.7%). The enrichment of organic molecules of molecular weights 150–300 from air where the predominant components possess molecular weights of 18 ($\text{H}_2\text{O}$), 28 ($\text{N}_2$), 32 ($\text{O}_2$), 40 ($\text{A}$) and 44 ($\text{CO}_2$) would appear much easier than that of the separation of the uranium hexafluorides. With the aid of a grant from the Swedish Board for Technical Development (project leader, Tekn. Dr. Hans Reinhardt), we have tried to build a suitable centrifuge. In the instrument constructed first the centrifuge element was built into the rotor of a three-phase motor, whose speed was regulated by varying the frequency. The construction had the attractive safety feature that the stator with its electrical windings lay as a mechanical shield around the rapidly rotating heavy rotor. The construction with a stiff axle led to a considerable strain on the bearings, however. Both air bearings and ball
Fig. 6. Gas chromatogram of volatile compounds from 1 Dufour gland of Camponotus ligniperda, worker ant. The chromatogram was run on the GC/MS combination instrument, utilizing a precolumn tube (cf. Bergström, 1973).

Fig. 7. Capillary gas chromatogram of volatile compounds from 1 Dufour gland of Camponotus ligniperda, worker ant. The chromatogram was run on a gas chromatograph, modified with a splitter-free intake system (cf. Ställberg-Stenhagen, 1972).

bearings were used but neither would allow runs for longer periods at speeds higher than 25 000 rpm. We finally gave up and after considerable rethinking, another design was adopted. This uses an air turbine drive system patterned after an American design. We hope to be able to test this device in the near future.

In most analyses, the volatile material has been subjected both to combined gas chromatography/ mass spectrometry and to capillary gas chromatography. It is now planned to combine a capillary gas chromatograph with a mass spectrometer of increased sensitivity (cf. Fig. 5).

The gas chromatogram in Fig. 6 was obtained using a standard gas chromatograph, attached to the mass spectrometer (LKB 9000). The gas chromatograph was equipped with a pre-column (Bergström,
Scheme 1. Normal procedure in the chemical and biological analysis of olfactory signals.

<table>
<thead>
<tr>
<th>Biological analysis in the field</th>
<th>Chemical analysis</th>
<th>Biological analysis in the laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preliminary observations¹</td>
<td>Separation⁴</td>
<td>Identification⁵</td>
</tr>
<tr>
<td>Isolation²</td>
<td>Experiments with natural secretions³</td>
<td>Experiments with model compounds³, ⁶</td>
</tr>
<tr>
<td>Experiments with natural secretions³</td>
<td>Experiments with natural fractions³</td>
<td>Interpretation of results⁷</td>
</tr>
<tr>
<td>Experiments with natural fractions³</td>
<td>Experiments with natural secretions³</td>
<td></td>
</tr>
<tr>
<td>Experiments with model compounds³, ⁶</td>
<td>Experiments with natural fractions³</td>
<td></td>
</tr>
</tbody>
</table>

1. Field and laboratory studies on behaviour and general biology. Preliminary chemical observations (smell, etc.).
2. Adsorption of odours or excision of glands. Direct degassing (pre-column) or solvent extraction.
3. Behavioural studies are a prerequisite, as well as construction and testing of experimental devices. Rearing of animals for laboratory experiments.
4. Mainly gas chromatography, which also gives structural information, but also thin-layer- and column (liquid) chromatography. The separation usually involves several steps, giving many fractions.
5. Mainly mass spectrometry, but also infrared spectroscopy, as well as microchemical methods, such as hydrogenation and ozonolysis. The identifications involve synthesis and/or preparation and characterization of reference compounds from natural sources.
6. Model compounds (synthetic and prepared from natural sources) are tested alone and in combination.
7. An evolutionary explanation of the results is sought for. This involves elucidating the physiological as well as the ecological function of the compounds and also their biosynthesis.

1973). A column packed with 2\% Silicone SE-30, "Ultraphase", on Chromosorb G 80–100 mesh, was used. Temperature was programmed with 4°C per minute from 80°C to 200°C. The material analysed was the contents of the Dufour gland from one worker ant of Camponotus ligniperda. The analysis of this material has been described in a recent report (Bergström & Löfgqvist, 1972). Fig. 7 shows the same material run on a capillary column (23 metres long, 0.25 mm diam.) OV-101 as stationary phase. A comparison between the two chromatograms reveals the higher resolution attainable, when using a capillary column with the intake system shown in Figs. 1–3.

The higher sensitivity of the mass spectrometer shown in Fig. 5 is due to its large magnetic radius (30 cm), which allows a higher accelerating voltage and larger slits for the same resolution as the smaller instruments.

Another possibility to improve the sensitivity lies in the use of a spectrophotometer with highly sensitive television camera tubes as detectors. The Westinghouse SEC tubes allow the integration over a period of time of several minutes. An efficient ion-light converter, such as the type described by von Ardenne (1962), von Ardenne et al. (1971) has to be used. Another possibility is to use a photomultiplier channel plate as an ion-electron converter, and let the electrons impinge on a suitable phosphor. The resulting optical image is transferred through fiber optics to the TV-tube. The limitations of such a system will be set by the noise levels encountered. The possibility of constructing a Hadamard transform mass spectrometer should also be considered.
The new infrared spectrophotometers of the pulse Fourier transform type would seem to be useful in analysing the effluent fractions from gas chromatographs (cf. Becker & Farrar, 1972).

During our work we have found that in order to achieve an ideal analytical process three conditions ought to be fulfilled. First, it is essential that the chemical analysis be as "closed" as possible. That is, the various analytical steps (see Scheme 1) should follow each other with a minimum of manipulations, in which material can be lost or changed in an uncontrolled way. The combination of gas chromatograph and mass spectrometer, as well as the use of splitter-free intake systems and pre-column tubes are examples of steps taken in this direction, which have been very helpful. Another measure would be to arrange various micro-chemical processes, such as hydrogenation, hydrogelysis and ozonolysis, to be performed directly on the gas chromatographic column (cf. e.g. Hoff & Feit, 1964; Beroza & Sarmiento, 1966; Beroza, 1970).

A second prerequisite for an optimal analytical procedure would be to have some steps of the chemical and biological analyses "coupled" (see again Scheme 1). The meaning of this is that the biological activity of the material should be tested at many steps in the analysis by direct or indirect methods. Directly in the field or in laboratory tests, indirectly through olfactometers or by electrophysiology. The "coupling" demands that animals are available in the field or through breeding and that the behavioural situations are known to some extent and can be reproduced.

Finally, and this is basically a problem of organization, several of the biological and chemical activities in the field and in the laboratory should, if possible, be pursued in parallel. This has led us to establish a close co-operation between biologist and chemist. The ecological station on Öland provides excellent conditions for such co-operation.

REFERENCES


Bergström, G. 1973. Studies on natural odoriferous com-
Application d'une Méthode Mathématique à l'Étude de Quelques Espèces du genre *Ophrys* L.

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RÉSUMÉ

Raynaud, C. (Laboratoire de systématique et de géobotanique méditerranéennes, Institut de Botanique, Montpellier, France.) Application d'une méthode mathématique à l'étude de quelques espèces du genre *Ophrys* L. ZOON, Suppl. 1: 83–91, 1973

Cette note n'est que la vérification, à l'aide d'une méthode mathématique, des résultats de l'étude de plusieurs populations d'*Ophrys*. Cette étude utilisait la méthode d'Anderson (1949). Dans les deux cas, les résultats sont semblables. La méthode mathématique permet de préciser la position de certains groupes d'individus.

L'origine hybride de l'*Ophrys luteola* Camus semble se confirmer; les conclusions obtenues sont comparées avec la classification évolutive de ce genre établie par B. Kullenberg (1961).

GÉNÉRALITÉS


De ce travail nous avions conclu que cette population d'*Ophrys* à sépales verts était formée de groupes d'individus, reliés entre eux par de nombreuses formes de passage. La présence, chez l'un de ces groupes, de caractères intermédiaires entre certains individus étudiés et d'autres appartenant à une espèce que nous n'avions pas récoltée, nous avait suggéré l'idée de l'origine hybride de ce groupe. Mais pour vérifier cette hypothèse, il fallait davantage de mesures portant sur des lots d'individus plus nombreux et aussi un plus grand nombre de caractères.

Les *Ophrys* à sépales roses étaient-elles aussi bien séparées dans l'ensemble de la population et là, nous soussomptions d'autres formes de passage que nous nous proposions de mettre en évidence.

Durant le printemps 1971, nous avons ajouté aux 90 individus récoltés l'année précédente 89 autres, soit un total de 179 pour la station de Montbazin, auxquels se sont ajoutés: 30 individus du lieu dit « La Paillade », 27 individus du lieu dit « Caunelle »¹, 38 individus du lieu dit « Fontfroide-le-Haut », 2 individus aux Sources du Lez.

Toutes ces stations sont situées dans la banlieue de Montpellier.

Ensuite, nous sommes allé récolter des plantes plus loin: 10 individus à Saint-Guilhem-le-Désert (Hérault), 23 individus à Saint-Martin-de-Londres (Hérault), 50 individus au Mas de l'Euzière (commune de Saint Félix-de-Palhères, Gard).

Nous avons ajouté des échantillons provenant de régions situées près de: Carcassonne dans l'Aude (16 individus), Montréal dans l'Aude (10 individus), Cadarache dans le Vaucluse (23 individus).

Enfin les excursions de la 99e session extraordinaire de la Société botanique de France, qui se sont déroulées en Loire-atlantique, nous ont permis de recueillir: 14 individus sur un côtéau calcaire près de Nantes, 7 individus à Belle-Île-en-Mer.

Sur chaque station, les fleurs ont été prélevées sur des individus pris au hasard, parfois même sur tous les individus rencontrés.

Ainsi avons-nous eu des plantes provenant de régions plus ou moins éloignées du Languedoc méditerranéen (cf. Tableau 1).

Les 429 fleurs (la première ou quelquefois la seconde de la hampe florale) ont toutes été prélevées sur des pieds différents. Sur ce matériel, à l'aide d'un compte-fil, nous avons mesuré et noté:

- longueur et largeur des sépales
- longueur et largeur des pétales

¹ La station de Caunelle est actuellement en voie de disparition et celle de La Paillade a totalement disparu.
Tableau I. Liste des stations étudiées, et nombre d'individus récoltés sur chacune d'elles

<table>
<thead>
<tr>
<th>Stations</th>
<th>Nombre d'individus récoltés</th>
</tr>
</thead>
<tbody>
<tr>
<td>Montbazin (Hérault)</td>
<td>179</td>
</tr>
<tr>
<td>Montpellier — La Paillade (Hérault)</td>
<td>50</td>
</tr>
<tr>
<td>Caunelle (Commune de Juvignac, Hérault)</td>
<td>27</td>
</tr>
<tr>
<td>Sources du Lez (Commune de Castelnau-le-Lez)</td>
<td>2</td>
</tr>
<tr>
<td>Fontfroide-le-Haut (Hérault)</td>
<td>38</td>
</tr>
<tr>
<td>Saint Guilhem-le-Désert (Hérault)</td>
<td>10</td>
</tr>
<tr>
<td>Saint Martin-de-Londres (Hérault)</td>
<td>23</td>
</tr>
<tr>
<td>Mas de l'Euzière (Commune de Saint Félix-de-Palhères, Gard)</td>
<td>50</td>
</tr>
<tr>
<td>Carcassonne, Commune de Villelalhens (Aude)</td>
<td>16</td>
</tr>
<tr>
<td>Montréal, Commune d'Arzens (Auds)</td>
<td>10</td>
</tr>
<tr>
<td>Centre nucléaire de Cadarache (Vaucluse)</td>
<td>23</td>
</tr>
<tr>
<td>Coteaux près de Nantes (Loire-atlantique)</td>
<td>14</td>
</tr>
<tr>
<td>Belle-Île-en-Mer</td>
<td>7</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>429</strong></td>
</tr>
</tbody>
</table>

forme des pétales-
largeur du labelle à l'état frais et à l'état sec-
largeur de la bordure jaune du labelle-
hauteur des gibbosités-
couleur des sépales-
couleur des pétales-
couleur du labelle-
longueur du gynostème-
couleur des loges des pollinies-
labelle entier ou labelle trilobé-
pilosité du labelle quand elle est abondante-
presence ou absence d'un appendice sur le labelle.

Ensuite, ces fleurs ont été décolorées dans les va-
peurs de gaz sulfureux, puis séchées, le labelle étant
au préalable détaché des autres pièces du périanthe
et le gynostème supprimé. Une fois les fleurs séchées,
les caractères quantitatifs, longueur et largeur, ont
été à nouveau mesurés sur les pièces mises à plat.

MÉTHODES EMPLOYÉES

Méthode d'Anderson

Avant d'appliquer la méthode de l'analyse factorielle,
dans un premier temps, nous avons traité tous les
caractères recensés ci-dessus par une méthode bio-
métrique inspirée de celle d'Anderson (1949) et qui
fut reprise par Stebbins et Ferlan (1956).

Les différentes caractères tant quantitatifs que
qualitatifs sont groupés en classes égales et chaque
classes est affectée d'une « valeur d'indice ». Ainsi
pour un caractère donné, chaque fleur se situera dans
une classe; cette fleur aura donc, pour ce caractère,
edue « valeur d'indice » qui lui sera propre. Un histo-
gramme pourra donc être tracé montrant la fré-
quence des individus pour chaque classe de cara-
tères.

À la fin, pour chaque individu, on fait la somme de
toutes les « valeurs d'indice » attribuées à chaque
caractère, on obtient ainsi un « indice global ». On
peut partir de là tracer un histogramme général.

Cette méthode nous a permis de déterminer, en
fonction des caractères pris en considération, 11
groupes au sein des populations d'Ophrys étudiées.

Ces groupes, notées en chiffres romains de I à XI,
ont pu être identifiés par rapport aux espèces
classiques du genre Ophrys:

I comprend les individus appartenant à l'espèce
*Helxina* Cav.; II, *litigiosa* Camus; III, *sphegodes Mill.
(synonymie *Ophrys sphegodes Mill.* = *Ophrys ari-
nifera Huds.*.) (s.l.); IV, *sphegodes Mill.* (synonymie
*Ophrys sphegodes Mill.* = *Ophrys arnifera Huds.*)
(s.s.); V, *sphegodes Mill.* subsp. *atra* (Lindl.) Mayer;
VI, *arachnitiformis* Gren. et Phil. (s.l.); VII, *arachni-
tiformis* Gren. et Phil. (s.s.); VIII, *scolopax Cav.*; IX,
insectifera L. (synonymie *Ophrys insectifera L.* =
*Ophrys muscifera Huds.*); X, *bertoloni* Moret.; XI,
*apifera Huds.*

Méthode d'analyse factorielle

des correspondances

Il nous a paru intéressant de confronter les résultats
obtenus par cette première méthode avec ceux obtenus
par une méthode plus moderne. Pour le faire, nous
avons soumis, à titre d'essai, toutes les données re-

Fig. 1. Répartition des « groupements » d'individus
(A, B, C, D, E) formés par les groupes I à XI, et des
variables selon les axes 1 et 2.
cueillies sur les 429 individus d’Ophrys étudiés à une analyse factorielle des correspondances. D’autres méthodes seraient peut-être plus appropriées au traitement de nos données; mais nous n’avions pas accès aux programmes correspondants. Par contre, nous disposons d’un programme d’analyse factorielle des correspondances établi par M. Roux et modifié par MM. David et Romanet. C’est grâce à ces deux personnes que cet essai a pu être réalisé sur l’ordinateur IBM 360-40 de l’Université des sciences et techniques de Languedoc.

D’une manière très générale, disons qu’une analyse factorielle permet d’obtenir sur un petit nombre d’axes de coordonnées nouvelles une image des points d’un ensemble dont les relations (ou « distances ») exactes ne pourraient être représentées que dans un espace à nombreuses dimensions. Ces axes nouveaux, permettant ainsi d’obtenir une image simplifiée (cf. Fig. 1), peuvent être considérés comme des combinaisons des axes originaux (à chacun desquels correspond un « facteur »). Nous ne leur donnerons ici que leur numéro (1, 2, 3 et 4) sans interprétation de leur signification.

Les détails de cette méthode ne seront pas traités ici; les aspects mathématiques ainsi que certaines applications sont, en effet, longuement exposés dans des travaux tels que ceux de Benzecri (1966, 1970), de Cordier (1965), de Lacoste et Roux (1972) et de Roman (1972).

« Groupements » définis par
l’analyse factorielle

Les caractères des individus pris en considération dans cette analyse sont les suivants: 1, rapport L/l des sépales divisé en 13 classes; 2, couleur des sépales, 9 classes; 3, rapport L/l des pétales, 12 classes; 4, couleur des pétales, 8 classes; 5, formes des pétales, 5 classes; 6, coefficient de convexité du labelle – largeur du labelle sec/largeur du labelle frais, 4 classes; 7, largeur de la bordure jaune du labelle, 3 classes; 8, taille des gibbosités du labelle, 3 classes; 9, division du labelle, 3 classes; 10, pili- sirité du labelle, 2 classes; 11, couleur du labelle, 3 classes; 12, couleur des loges des pollinies, 2 classes; 13, caractère de l’appendice apical, 3 classes; 14, longueur du gynostème, 3 classes.

Au total 14 caractères auxquels correspondent 72 classes. Mais il convient de préciser que nous avons,

dans l’analyse factorielle, considéré tous les caractères comme ayant le « même poids ».

Le tableau des données analysées est une matrice de 0 ou de 1, où le 1 indique pour chaque individu la classe du caractère considéré.

Quatre diagrammes successifs ont été tracés selon les couples d’axes de coordonnées 1-2, 1-3, 2-3 et 3-4; sur chacun de ces diagrammes, chaque individu est matérialisé par un point, ainsi d’ailleurs que chacune des 72 classes. Sur ces diagrammes certains « groupements » sont assez bien individualisés; nous les avons délimités de manière empirique, puis, nous les avons comparés aux groupes déjà obtenus par la méthode biométrique.

C’est le diagramme tracé selon les axes 1 et 2 (Fig. 1) qui permet de mettre en évidence la meilleure individualisation de ces « groupements ».

Plusieurs faits ressortent de l’examen de ce diagramme.

a) Certains « groupements » ne comprennent que des individus d’un seul groupe; c’est le cas des « groupements » A, C et E qui ne sont composés respectivement que par des individus des groupes V, IX et I, caractérisés par les espèces sphegodes subsp. tratra, insectifera et lutra;

b) le « groupement » B comprend des individus des groupes XI et VIII représentant les espèces api- fera et scolopax; toutefois les individus du groupe XI (api fera) se localisent dans une petite partie bien précise du « groupement »;

c) le « groupement » D comprend des individus des groupes II, III, IV, VI, VII et X soit respectivement les espèces ligiosa, sphegodes s.l., sphegodes s.s., arachnitiformis s.l., arachnitiformis s.s. et bertoloni; toutefois l’on peut observer en son sein une certaine organisation entre les individus de certains de ces groupes quand on agrandit le diagramme (cf. Fig. 2 et 3).

En effet, la figure 2 nous permet de constater que les individus du groupe II (O. ligiosa) ont une répartition différente de celle des individus du groupe IV (O. sphegodes s.s.); on peut en effet considérer deux « sous-groupements » : le « sous-groupement » D_1 comprenant la majorité des individus du groupe II (O. ligiosa) et le « sous-groupement » D_2 comprenant la majorité des individus du groupe IV (O. sphegodes s.s.).

Le « sous-groupement » D_1 comprend les classes aux caractères suivants : pétales vert-jaune, labelle à faible bordure jaune, couleur vert clair des sépales, pétales oves et pétale oves-elliptiques (ces deux

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1 Centre d’études phytosociologiques et écologiques L. Emberger, Montpellier.
dernières classes possèdent des coordonnées très voisines, presque confondues et sont donc difficilement séparables.

Le « sous-groupement » D2 comprend des caractères tels que pétales vert foncé, absence de bordure jaune et les caractères propres au groupe IV, c'est-à-dire, pétales de forme ovale-anguleuse et gibbosités bien développées.

Cette figure nous montre également la position intermédiaire qu'occupe le groupe III (O. sphegodes s.l.) par rapport aux groupes II (O. litigiosa) et IV (O. sphegodes s.s.). Les individus constituant ce groupe se rencontrent principalement sur la frontière des deux « sous-groupements » D1 et D2 ainsi que le long de deux prolongements émis par le « sous-groupement » D2 à l'intérieur du « sous-groupement » D1. Il convient de préciser ici que la définition de ce groupe avait été très délicate à l'aide de la méthode biométrique.

De même, la figure 3 montre que l'on peut difficilement individualiser les groupes VI et VII, appartenant tous deux à l'espèce arachnitiformis, à partir de cette analyse factorielle des correspondances telle qu'elle a été faite. On peut, en effet, constater que les individus du groupe VII (O. arachnitiformis s.s.) délimitent une surface autour de laquelle se situe la majorité des individus constituant le groupe VI (O. arachnitiformis s.l.).

A la suite de cet essai, il nous a été possible de noter une similitude certaine entre les résultats obtenus par cette analyse et ceux obtenus par la méthode d'Anderson; c'est le cas pour les espèces lutea, litigiosa, sphegodes s.s., insectifera, bertolonii, caract-

térisées respectivement par les groupes I, II, IV, IX et X. II nous a été également possible de préciser la position de certains groupes jusqu'alors demeurée très floue (cas de l'espèce *sphegodes* s.l. : groupe III).

Des divergences entre les résultats obtenus par les deux méthodes apparaissent néanmoins :

a) Les individus du groupe V (*O. sphegodes* subsp. *atrata*) étaient inclus, dans la méthode biométrique, avec ceux du groupe IV (*O. sphegodes*); ils s'en trouvent ici séparés; ceci parait être dû au fait que tous les caractères sont considérés dans l'analyse factorielle comme ayant le même « poids ».

b) Le phénomène inverse se produit pour les individus appartenant à l'espèce *apifera* (groupe XI) qui sont, dans l'analyse factorielle, regroupés avec ceux appartenant à l'espèce *scolopax* (groupe VIII), alors qu'ils étaient éloignés dans la méthode biométrique pour la même raison de « force » dans les caractères choisis.

Ceci souligne bien l'importance de certains caractères par rapport à d'autres et le besoin qu'il y a d'établir une hiérarchie entre eux, point qui demeure très délicat, car il est le privilège du jugement de chacun.

**Comparaison entre nos résultats et la classification de Kullenberg (1961) :**

le problème des *Ophrys arachnitiformis, litigiosa* et *sphegodes*.

Dans la classification de Kullenberg, deux grands groupes d'*Ophrys* sont discernables. Ils sont séparés par une barrière offensive importante. Sur la figure 4 cette séparation est représentée par le double trait horizontal.

En bas, nous avons des espèces caractérisées par les pièces du périanthe vertes; en haut, des espèces avec des pièces du périanthe roses. Cette division sommaire n'est pas parfaite, mais, vue ainsi, elle est semblable à la classification de Godfrey (1917) qui divisait les *Ophrys* en deux groupes :

A. Eu-Sepalaæ : sépales verts, herbacés.

B. Pseudo-Sepalaæ : sépales roses ou blancs, pétaloïdes.

Nous pensons que la caractère couleur des sépales ne peut constituer une séparation valable. Ceci surtout pour l'espèce *sphegodes* s.l. où des individus peuvent présenter des sépales roses. Or, dans la classification de Kullenberg, le passage entre les deux groupes que nous mentionnons plus haut se fait justement au niveau de cette espèce. Ce passage aboutit d'ailleurs à l'*Ophrys fuciflora* (Crantz) Moench.

L'*Ophrys arachnitiformis* représentée chez nous par les groupes VI et VII n'est pas mentionnée dans ce tableau. Cette espèce est étudiée actuellement par Kullenberg afin d'être intégrée dans son système de classification évolutive.

Si cette *Ophrys* est, comme beaucoup d'auteurs le pensent, issue d'une origine hybride entre les *Ophrys* du groupe *sphegodes* et *fuciflora*, il faut que les pollinisateurs de ces deux plantes soient les mêmes. Or pour le moment Kullenberg a noté la présence sur *O. sphegodes* d'Hyménoptères du genre *Andrena* et sur *O. fuciflora* d'Hyménoptères du genre *Eucera*, ce qui normalement exclut toute hybridation.

Mais les multiples variations du groupe *sphegodes* ont pu faire apparaître une plante dont les fleurs exhalent un composé volatile attirant accidentellement un pollinisateur d'*O. fuciflora* ou inversement. Il se pourrait aussi que les variations extrêmes des caractères floraux de l'*O. sphegodes* et de l'*O. fuciflora* aient fait converger les caractères des labelles vers une structure commune avec un composé chimique mélange des deux, qui troublerait ainsi l'odorat de l'insecte. Nous n'exposons là que des suppositions.

Nous avons pu établir par le calcul le lien existant entre *O. sphegodes* (groupe IV) et *O. arachnitiformis* (groupe VI et VII), mais le manque d'individus appartenant à l'espèce *fuciflora* ne nous a pas permis de faire la même opération pour cette dernière espèce.

Nous considérons pour le moment *O. arachnitiformis* comme une espèce distincte de *O. sphegodes*, mais rattachée à cette dernière par de nombreuses formes de passage.

Le problème concernant l'*Ophrys litigiosa* Camus (groupe II) est pour nous plus intéressant. Nous avons vu comment, sur le figure 2, les plantes appartenant à ce taxon apparaissaient groupées dans une surface possédant une partie tangente avec celle de l'*Ophrys sphegodes* Mill. (groupe IV). Selon les mesures que nous avons faites, *O. litigiosa* pourrait avoir une origine hybride entre cette *Ophrys* et l'*Ophrys lutea* (groupe I), vers laquelle certains individus semblent tendre par leurs caractères.

A l'heure actuelle l'*Ophrys sphegodes* possède une aire de répartition géographique étendue à la totalité de la France et dépassant largement ses frontières. Dans la région méditerranéenne les individus pouvant être rattachés à cette espèce possèdent des ca-
Caractères floraux engagés dans la différenciation de la forme :

1. Parfum
2. Contour
3. Structure épidermique du labelle
4. Taille de la fleur
5. Relations allométriques
6. Couleur du labelle et des sépales

Fig. 4. Evolution hypothétique du genre *Ophrys* L.

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- **Barrière tactile**
- **Relation phylogénétique supposée**

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*Eucera*: nom de l'insecte pollinisateur
ractères relativement éloignés du type. Si nous nous
éloignons des stations à influence strictement médi-
terranéenne, les individus deviennent plus conformes
au type de l’espèce.

Etant donné l’ampleur de la variation, il est dif-
ficile de dire où s’arrêtent vraiment les caractères
faisant appartenir un Ophrys à l’espèce sphaegodes.
Dans la population de Montbazin se trouvent des
individus aux caractères intermédiaires entre les
espèces sphaegodes et littigiosa.

Si nous observons la répartition géographique de
l’Ophrys lutea, nous voyons que son aire géo-
graphique plus restreinte est davantage méditer-
ranéenne que celle de l’Ophrys sphaegodes. Elle reste
inféodée au bassin méditerranéen. Dans notre région
Cette plante est stable, même dans la forme de la
tache du labelle. Pourtant nous pouvons récolter sur
les stations de Montbazin et de Canéelle près de
Montpellier, des plantes aux caractères intermédiai-
res entre Ophrys lutea et Ophrys littigiosa. Ces indi-
vidus ne sont pas aussi nombreux que les formes in-
termédiaires entre Ophrys littigiosa et Ophrys spe-
ghodes, mais ils se distinguent très nettement et,
sur nos diagrammes, ils sont situés dans la partie gauche
du groupe II et le long du prolongement émis par ce
groupe en direction du groupe I (cf. Fig. 2).

Ces différentes observations nous ont conduit à
penser que l’Ophrys littigiosa pourrait être un hybride
lointain entre O. sphaegodes typique et O. lutea. À
l’heure actuelle ces plantes ont des aires bien distinc-
tes (tand qu’il s’agit de l’O. sphaegodes typique), mais
en a-t-il été toujours ainsi?

En tenant compte de fait que l’O. sphaegodes pos-
sède une aire géographique plus septentrionale, on
peut facilement penser que lors de l’expansion
maximale des glaciers, au Quaternaire, cette Ophrys
est descendue et est venue rejoindre l’O. lutea. Les
limites de leurs aires butaient alors aux rivages de la
Méditerranée. Ces deux plantes au contact l’une de
l’autre purent donner un hybride grâce à l’action du
même insecte pollinisateur.

Kullenberg sépare ces deux Ophrys par une bar-
rrière tactile : l’insecte se pose sur le labelle de
l’Ophrys lutea la tête en bas, alors qu’il pénètre sous
le gymnoste de l’Ophrys sphaegodes avec la tête en
avant. Cette position de l’insecte pollinisateur avait
déjà été étudié par Godfery qui, en 1928, proposait
sa seconde classification du genre. Cette fois il
divisait le genre en deux sections, avec, comme ca-
ractère distinctif, la position des pollinies au mo-
ment du départ de l’insecte. Soit :

Section Eu-Ophrys : pollinies fixées sur la tête
Section Pseud-Ophrys : pollinies fixées sur le
dernier segment abdominal.

C’est dans cette dernière section que se trouve
l’Ophrys lutea.

Nous avons personnellement observé quelques pol-
linisateurs et nous avons eu une fois la surprise de
voir sur un labelle d’Ophrys sphaegodes s.l., un insecte
dans la position qu’il devrait avoir sur un labelle
d’Ophrys lutea.

CONCLUSION

En conclusion on peut supposer que de grands bou-
leversemens comme les glaciations du Quaternaire
ont pu faciliter la rencontre d’espèces jusqu’alors
séparées et provoquer la création de nouveaux
hybrides. Une fois ce nouvel hybride créé, il a pu à
son tour, par croisement avec les parents, donner
des individus introgressés. Mais cette opération,
facilitée par la position identique que prend la polli-
nisante sur le labelle des deux partenaires, fut cer-
tainement plus difficile avec l’Ophrys lutea chez
laquelle l’insecte a une position la plus large du temps
inverse.

Lors de la remontée des glaciers, Ophrys lutea est
restée dans les régions où nous la rencontrons ac-
tuellement. Le réchauffement du climat a éloigné
l’Ophrys sphaegodes s.s., des stations trop méditer-
nanéennes. Les individus formés par introgression ont
pu alors se distribuer le long du parcours.

Ou bien, on peut imaginer que la remontée de
l’Ophrys sphaegodes fut accompagnée de celle de
l’Ophrys littigiosa et que des individus introgressés
peuvent se former au fur et à mesure de cette migra-
tion plus ou moins commune. Camus (1927), en
note infrapaginale, cite une observation de Walter,
d’après lequel la remontée de l’Ophrys littigiosa s’est
faite par la trouée de Belfort pour aboutir sur le
flanc est des Vosges et en Alsace. En effet cette
plante ne se rencontre que sur le flanc ouest de ce
massif.

Si on observe les aires de répartition géographique
de l’Ophrys littigiosa, aires établies par Nelson, on
s’aperçoit que cet auteur considère comme douteuse
la présence de cette plante dans la vallée du Rhône
ainsi que dans le Bassin parisien. Pour lui les sta-
tions françaises de cette Ophrys sont limitées au
bassin méditerranéen et aux régions du Jura et
d’Alsace.
Il semblerait donc que l'origine de l'Ophrys litigiosa soit bien le bassin de la Méditerranée.

Nous n'avons pas rencontré cette plante sur les stations où poussait l'Ophrys sphegodes typique. Il semble donc que, mise à part la région vosgienne, l'aire de l'Ophrys litigiosa soit pour la France limitée à la région méditerranéenne.

Pour toutes ces raisons, nous pensons finalement que l'Ophrys litigiosa est une espèce rattachée au grand groupe sphegodes, mais qu'elle a certainement une origine hybride issue d'un croisement ancien entre O. sphegodes et O. lutea. Ce croisement fut certainement accidentel.

Le cas de l'Ophrys arachnitiformis est certainement plus complexe, bien que le problème puisse se poser de la même façon. Il n'en reste pas moins que, pour cette dernière espèce, si elle peut par ses caractères floraux être considérée comme un intermédiaire possible entre O. sphegodes et O. fuciflora, l'hypothèse d'un croisement reste difficile du fait de la différence des pollinisateurs.

Ce ne sont là bien sûr que des hypothèses et seuls des croisements artificiels pourraient les infirmer ou les confirmer, bien qu'étant donné le nombre et la variabilité des caractères, les combinaisons au niveau des gènes soient multiples et que l'hybride artificiel puisse bien ne ressembler à aucune des plantes connues.

**BIBLIOGRAPHIE**


The Ultrastructure of Mycorrhiza in Ophrys insectifera

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ABSTRACT

Hofsten, A. von. (Institute of Physiological Botany, Uppsala University, Uppsala, Sweden.) The Ultrastructure of Mycorrhiza in Ophrys insectifera. ZOON, Suppl. 1: 93–96, 1973

The ultrastructure of the endotrophic (intracellular) mycorrhiza of the orchid Ophrys insectifera has been studied in the electron microscope. The pictures illustrate the appearance of the fungal infection and show a balanced state of symbiosis. The fungal cells are surrounded by a double cell wall and sometimes small vesicles have been developed from the outer cell wall. No isolation has been made of the orchid fungus on the roots of Ophrys insectifera.

INTRODUCTION

The occurrence of mycorrhiza is an exceedingly widespread phenomenon in the plant kingdom. Almost all land plants are provided with mycorrhiza, and the forest botanist, Hartig, made the first observations of fungal attack on roots on conifers in 1851. In 1909 Burgeff published many research papers, where he discussed the occurrence of mycorrhiza in the Orchidaceae, and Bernard isolated the first orchid fungus in 1903. The term mycorrhiza was coined by Frank (1887) from the Greek, mykes meaning fungus and rhiza, root.

At the Institute of Physiological Botany in Uppsala, Professor Elias Melin has been working on problems of mycorrhiza for almost fifty years. He described several forms of mycorrhiza in his book "Experimentelle Untersuchungen über die Konstitution und Ökologie der Mykorrhizen von Pinus silvestris L. und Picea abies L. Karst" (Melin, 1923). He defined the ectotrophic (ektos: outside) mycorrhiza that occur among our forest trees as intercellular. The endotrophic (endos: inside) type which is common in the roots of many orchids is defined as intracellular. Dr Harley (1969) recently wrote a book, "The Biology of Mycorrhiza" where he summarizes old and new results of anatomical and physiological problems concerning the mycorrhiza complex.

As far as we know today, all orchids live in symbiosis with a root fungus. The green orchids Platanthera (Bernard, 1903) and Orchis (Burgeff, 1954) are mostly infected by the imperfect genus Rhizoctonia. Gäumann et al. (1950) studied the mycorrhizal fungi in Orchis, and found mostly Ascomycetes and Basidiomycetes. The true saprophytes, Neottia, Corallorrhiza and Epipogium live in symbiosis with Hymenomycetes. The infection occurs somehow through root hairs of the adult plant, and the fungus penetrates the plant from the soil, possibly attracted by substances secreted by the plant (Werner, 1950). The infecting hyphae are thin, and become much thicker in the host cells of the roots. Some lignin-deestroying symbiont, e.g. Fomes, destroys the cell walls of the host orchid and penetrates and multiplies in all directions in the root cells.

Some scientists postulate (McDougal & Dufrenoy, 1944) that orchids are parasites on fungal mycelium. The hyphae contain protein, glucogen and fat as a good source of nutrient for the plant. The hyphae of the fungus may also help the plant in uptake of nitrogen, phosphorus and other mineral elements from the soil. On the other hand the fungus obtains sugar and vitamins from the plant. Melin (1923) and Björkman (1949) characterize the phenomenon of mycorrhiza as a symbiotic relationship where the two partners live in a state of physiological balance.

Orchid seeds are very small and a plant sometimes produces up to one million seeds. A small germ is surrounded by a single-layered seed coat and for a good germination the seed must be infected by a fungus. The fungus penetrates through the basal cells of the germ and uses the starch in the seed as nutrition for developing. In some dry soils where there is a lack of fungal hyphae the germination of orchids is very poor.

This paper will show the ultrastructure of the mycorrhiza in Ophrys insectifera. Some new infor-
tioning, and the sections, about 300 Å, were post-stained with Pb and UAe. The specimens were examined in an Akashi TRS-50 electron microscope with a three-stage magnetic lens at a voltage of 50 kV, and a Siemens Elmiscoop 1 at an accelerating voltage of 60 kV equipped with a double condensor.

RESULTS

When roots of Ophrys insectifera are infected with a fungus the host cells are completely invaded by the endophyte. The fungal hyphae multiply into small cells bya budding process, similar to that of yeast. Figure 1 illustrates the endotrophic mycorrhiza in Ophrys insectifera at a low magnification. The fungal cells vary in size from 2–10 μm, and new cells are formed by budding (see B.C.). The cell wall between the cortical cells of the host is rather thin and holes in the secondary cell wall may facilitate new infections of the fungus into neighbouring cortical cells (see arrow Fig. 1). The fungal cells are surrounded by a double-layered cell wall (CW) measuring about 1 200 Å (Fig. 2). The cell wall con-

MATERIAL AND METHODS

Roots of Ophrys insectifera with mycorrhiza were collected in September 1967, near the Ecological Station at Ölands Skogsby, Öland, Sweden, where Professor B. Kullenberg helped me to select orchids from an over-flowering stage. The roots were rapidly washed in a veronal-acetate buffer with pH 7.2 to remove adhering soil and cut in 2 mm small segments. These were first prefixed in 5% glutaraldehyde and the roots of Ophrys were then postfixed in 2% OsO₄. The cell material was thereafter stained in 2% uranylacetate, dehydrated and embedded in Epon, according to a method described earlier by the author (v. Hofsten, 1969).

An “Ultrotome” microtome (LKB, Stockholm), equipped with diamond knives, was used for sec-

![](image1)

**Fig. 1.** Electron micrograph of fungal cells in the roots of Ophrys insectifera. BC = budding cell. CW = cell wall. Arrow indicates a hole in the cell wall.

![](image2)

**Fig. 2.** Fungal cells with double-layered cell walls (CW). The cytoplasm of the host (Ophrys) contain mitochondria (M) and collapsed plastids (P).
sists of two electron-dense layers of 400 Å and between these is an electron-transparent zone of 400 Å. The host cell contains numerous mitochondria (M) but the plastids (P) have almost collapsed after infection and digestion by the fungus.

Figure 3 shows some fungal cells with septa (S). These are formed from the inner electron-dense layer of the cell wall and they measure about 400 Å in diameter. In one of the cells a pore (P), developed to facilitate a cytoplasmic flow between the cells in the hyphae. Nuclear vacuoles (NV) are seen in almost every cell. Figure 4 illustrates a fungal cell in the digestion layer at a high magnification. The cell wall is double layered, and small vesicles have developed near the outer electron-dense layer of the cell wall (see arrows, Fig. 4) showing the contact between the host and the fungus. The cytoplasm contains many vacuoles (V) and is packed with glycogen (G). This figure shows that the phenomenon of mycorrhiza in Ophrys is a balanced state of symbiosis and not a pronounced parasitism.

The imperfect genus Rhizoctonia is supposed to attack green orchids. In my work with Ophrys insectifera I have not tried to isolate the fungal partner, but from the electron micrographs published here it is very likely that the fungus belongs to Rhizoctonia.

**ZUSAMMENFASSUNG**


**REFERENCES**


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Fig. 3. Fungal cells with nuclear vacuoles (NV), septa (S), one with a pore (P).
Fig. 4. A fungal cell with double-layered cell wall (CW), vesicles at the outer layer (arrows), glucogen (G) and vacuoles (V).
The Hedgehog *Erinaceus europaeus* and its Flea *Archaeopsylla erinacei*

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**ABSTRACT**


On the Baltic island of Öland a population of the flea *Archaeopsylla erinacei* (Bouché) was studied with regard to seasonal abundance, dispersal, and reproduction in relation to seasonal activity and breeding of its host *Erinaceus europaeus* L.

49714 specimens of hedgehog fleas were obtained from 385 hedgehogs, 79 of which were examined at least twice. The seasonal fluctuations of the mean infestation are given for three categories of animals: males, breeding females and non-breeding females. The reproductive cycle of the flea indicates that it breeds only in the nests of breeding female hedgehogs. After a short development adult fleas emerge in August, so that the young hedgehogs are infested before they finally leave the nest. The fleas have a great ability to disperse and there is a considerable exchange of fleas between the host specimens.

**STUDY AREA**

Hedgehogs were studied in a region of about 50 km² on Öland, an island in the Baltic. All the animals were captured in 11 smaller areas with a total area of about 5 km² (Fig. 1). Most areas include hamlets with gardens, roads and adjacent arable land. Exceptions were No. 9—a forest without settlement, and No. 5—about half the area covered with forest.

Only a few hedgehogs were captured outside the 11 areas.

**CLIMATE AND ENVIRONMENT**

The spring is late and cold on Öland due to the influence of the Baltic Sea. For the same reason, the autumn is long and warm. A further reason for the warm autumns is that the bedrock consists of superficial limestones that store solar energy. Summer is usually warm and dry.

Lime soils with high organic content dominate in the study area which supports a luxuriant vegetation. These parts of the island are old grazing land, nowadays forming a mosaic of arable land, enclosed pastures, grazing land with shrubs and trees, and dense deciduous woods, often with rich shrub and/or herb strata.

Farms are subdivided into small fields, usually enclosed by stone-walls flanked by shrubs or trees. This environment offers the hedgehog numerous sites for breeding and hibernation.

The variation of the study area favours the hedgehog population, which on Öland is rather dense.

**METHODS FOR CAPTURE OF HEDGEHOGS AND COLLECTION OF FLEAS**

Hedgehogs were captured in four periods (of 4–9 days) each year in the summers of 1969–1970 (Fig. 2).
hunting hedgehogs for some hours around twilight. Captured specimens were kept separately in plastic bags, until they were transferred the same evening to separate cages. The masonite walls of the cages were dug about 10 cm into the ground, so that fleas could not spread between the animals.

The hedgehogs with their ectoparasites were anaesthetized with diethyl ether the day after the capture and fleas and ticks were collected. The fur on the ventral side and the head had to be carefully searched for ectoparasites, while fleas sitting between the spines were easily removed when the anaesthetized animal was handled.

The hedgehogs were marked with a numbered metal clamp (obtained from the firm “America”, DK-7700 Tisted, Denmark) in the right ear. This mark was not visible in rolled-up hedgehogs. The right forefoot of the hedgehog was therefore marked with red nail-varnish, lasting for about 2 weeks, to avoid duplicate examinations in a capture period.

After the weight had been recorded, the hedgehogs were released <25 m from the spot where they had been captured 24 hours earlier.

**NUMBERS OF HEDGEHOGS**

385 hedgehog specimens were captured, 79 of them being recaptured one or more times (see Löfqvist, 1973a, 1973c) (Fig. 2).

The age of the hedgehogs was not determined, but
it was found (Löfqvist, 1973a) that bodyweight can be used for separating juvenile hedgehogs (yearlings) from adults of both sexes. Subadult hedgehogs could not be distinguished from adult animals by their weight.

LENGTH OF THE ACTIVE SEASON OF HEDGEHOGS

The hedgehogs hibernate in a nest usually built of straw and leaves (Morris, 1970). Hibernation starts when the temperature in the nest falls below 14.5-17°C (Herter, 1938, pp. 123-134), i.e. at a mean temperature in the day of 8-10°C (Herter, op. cit.). At these temperatures the animals rest in a lethargic stupor. Hibernation proper begins when the nest temperature falls below 14.5°C (Herter, op. cit.). The physiology of the hibernation and its rhythmic, intermittent nature have been studied by numerous workers whose findings have been reviewed by Morovsky (1971).

The hedgehogs wake up when the nest temperature rises above about 16-17°C (Herter op. cit.).

On Öland, the mean day temperature in May was 8-9°C during the period 1901-1930 (Ångström, 1946). The same temperature conditions were registered for October. This suggests that hedgehogs are usually active on Öland from early May to the end of October.

There are certain differences in the seasonal activity between male and female hedgehogs. Males were more numerous in the first capture period and to some extent also in the second one, while the females were dominant in the third and still more the fourth period. The reason is probably that during the rutting time in the first two periods the males are very active and the chance of capturing a male was greater than that of capturing a female. After rutting, which lasts until the beginning of July, the males live as solitaries and increase in weight very rapidly (Löfqvist, 1973a). By mid-August they had become fat and the decreasing numbers caught show that they undertook little movement in the region. On the other hand, females which had young in the season start to increase their weight in the middle or at the end of August (Löfqvist, 1973a). They are therefore active later in the autumn than the males. Juvenile animals are also active until very late in the season.

Fig. 3. Mean infestation rates of the hedgehog flea on adult and subadult male hedgehogs captured for the first time. The figures indicate the number of samples.

REPRODUCTION OF HEDGEHOGS

It was found that on Öland female hedgehogs were only rarely fertile as subadults, i.e. in their second summer (Löfqvist, 1973a), whereas this has been reported as common in England by Deanesly (1934). According to Allanson (1934) male hedgehogs are also fertile in their second summer in England. The maturation of male hedgehogs was not studied in our material.

The minimum gestation time in hedgehogs is 31 days (Morris, 1961). The average number of young is about four (see Löfqvist, 1973b). The young eat solid food from 21 days (Herter, 1938) and 25-26 days of age (Morris, op. cit.). At this age the young begin to follow their mother on her evening foraging sallies (Herter, op. cit.). When the young are 1-1½ months old they are weaned by their mother from whom they become increasingly independent (Herter, op. cit.).

We observed breeding hedgehogs from early July
to mid-August. Only a few females were found to breed outside this period.

Breeding female hedgehogs were identified by the appearance of their nipples.

**INFESTATION RATES OF FLEAS ON HEDGEHOGS IN SUMMER**

The total number of hedgehog fleas collected from the hedgehogs was 49,714. Only 10 specimens of other flea species were found. The mean numbers of hedgehog fleas collected on the various occasions was calculated for (1) adult and subadult male hedgehogs (Fig. 3), (2) female hedgehogs breeding in the season (Fig. 4), and (3) adult and subadult females not breeding in the season (Fig. 5). The numbers of fleas collected in 1970 were generally lower than in 1969. Similarly, the number of fleas collected from the male hedgehogs in September was high in 1969, rather low in 1970 and intermediate in 1971. One reason may be that collecting fleas in 1969 and 1970 may have reduced the reproductive potential of the population and thus their numbers late in 1970.

Therefore, fluctuations in the infestation of fleas on hedgehogs are best studied in the 1969 material. That year all three categories of host animals had the lowest number of fleas in July (Figs. 3–5). Breeding females had a much lower mean number (9.0) than males (45.5) or non-breeding females (24.0). The mean number of fleas in August was highest for breeding females (226.8), intermediate for males (158.6) and lowest for non-breeding females (98.2). The data for 1970 are similar. Similar tendencies were apparent in Zlotorycki’s (1955) material from Poland.

A fuller interpretation of the material will be given in a later paper.

**REPRODUCTION OF THE FLEAS**

The reproduction of the fleas can be followed in the oocyte development. Ripening oocytes distend the
Table 1. The mean length of female fleas

<table>
<thead>
<tr>
<th>Host category</th>
<th>May-June</th>
<th>July</th>
<th>August</th>
<th>September</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\bar{X}$</td>
<td>$n$</td>
<td>$\bar{X}$</td>
<td>$n$</td>
</tr>
<tr>
<td>Ad. and s. ad. males</td>
<td>1969</td>
<td>82.6</td>
<td>16</td>
<td>81.6</td>
</tr>
<tr>
<td></td>
<td>1970</td>
<td>87.1</td>
<td>28</td>
<td>85.2</td>
</tr>
<tr>
<td>Ad. and s. ad. non-breeding females</td>
<td>1969</td>
<td>82.9</td>
<td>13</td>
<td>83.6</td>
</tr>
<tr>
<td></td>
<td>1970</td>
<td>87.6</td>
<td>12</td>
<td>84.5</td>
</tr>
<tr>
<td>Ad. and s. ad. breeding females</td>
<td>1969</td>
<td>81.8</td>
<td>8</td>
<td>78.6</td>
</tr>
<tr>
<td></td>
<td>1970</td>
<td>85.4</td>
<td>5</td>
<td>80.5</td>
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<tr>
<td>Male yearlings</td>
<td>1969</td>
<td>85.6</td>
<td>4</td>
<td>78.9</td>
</tr>
<tr>
<td></td>
<td>1970</td>
<td>78.9</td>
<td>2</td>
<td>77.2</td>
</tr>
<tr>
<td>Female yearlings</td>
<td>1969</td>
<td>75.8</td>
<td>3</td>
<td>77.6</td>
</tr>
</tbody>
</table>

Note: The values are expressed in Leitz ocular units (1 unit = 0.033 mm). The values given are mean values of mean values calculated for samples from each hedgehog. For hedgehogs having a large number of fleas the mean value is based on measurements of 50 fleas.

abdomen, increasing the body length. Female fleas from adult/subadult male hedgehogs and from breeding or non-breeding female hedgehogs in the months May–June and July (1969 and 1970) did not differ as regards body length (Table 1). The fleas from male hedgehogs and from non-breeding females had the same size in August as in the two previous capture periods. Breeding female hedgehogs, however, had fleas of a smaller size, as also had, in general, the juvenile hedgehogs. In September, all mean values were low and there were no differences in the mean sizes of the fleas from the five host categories.

Three circumstances indicate that the fleas reproduce only in the nests of female breeding hedgehogs: first, the small size of the fleas from such animals and young in contrast to the larger size of fleas from males and non-breeding females in August; second, the abundance of fleas on breeding females in August; third, the rapid increase of fleas on breeding females from July to August. The size of the fleas also indicates that the fleas emerge before the young leave the nest. As the young follow the female on her foraging excursions from the age of 3–4 weeks until they are 1-1½ month old, the development of the flea from egg to imago seems to be short, probably 3–4 weeks as in many other flea species (Cotton, 1970a; 1970b; Rothschild & Hughes, 1970).

The oocyte development of the hedgehog flea will be discussed elsewhere.

DISPERSal OF THE FLEAS

As can be seen from Table 1, there is a rapid exchange of fleas between the various categories of hedgehogs. In August, newly emerged fleas were mainly observed on breeding female hedgehogs and on their young. But from the middle of September

![Fig. 6. Flea re-infestation of adult and subadult male hedgehogs after intervals of about a month. For comparison the mean infestation rates of males captured for the first time are given.](image-url)
they predominate and are abundant (Figs. 3–5) on all categories of hedgehogs (Table 1).

The rapid dispersal of the fleas is also evident from the re-infestation of cleaned adult and sub-adult male hedgehogs, recaptured in the next period (Fig. 6). When comparing the infestation rates of such animals with those of animals captured for the first time, the mean value differs only once in seven observations. The interval between the capture periods was about 1 month. It may also be noted that male hedgehogs have no nests in the summer, neither do they live together with breeding females for any considerable time (P. Morris, pers. comm.). The dispersal of fleas must be considerable in the summer season.

DISCUSSION

Most fleas breed in the nest of their hosts, which they manure with their excrements. Rothschild et al. (1964a, 1964b, 1966, 1970) found that the defecation rate of the flea *Spilopsyllus cuniculi* (Dale) increased to a very high level at its breeding time. The dried excrements contain much blood and form the main food resource for the larvae.

The nesting habits of hedgehogs in the summer are incompletely known. The breeding nests of the females were described by Lüttich (1928). The young stay in the nest until they are 3–4 weeks old (Herter, 1938; Morris, 1961). In the summer adult and sub-adult males and non-breeding females are nomadic according to P. Morris (pers. comm.).

It appeared that the hedgehog flea reproduces only in the nests of breeding female hedgehogs. It remains to be shown whether flea reproduction is released by host hormones in the same way as was shown for the rabbit flea, *Spilopsyllus cuniculi* (Mead-Briggs & Rudge, 1960; Mead-Briggs, 1964, Rothschild & Ford, 1964a, 1964b, 1966; Rothschild, Ford & Hughes, 1970).

Unlike many other fleas, the hedgehog flea emerges shortly after development. Fresh fleas are found in great numbers on male and female hedgehogs of all ages from the middle of September or earlier. Evidently, the dispersal of the fleas through the hedgehog population is more extensive than in most other flea species.

The re-infestation of rodents able to visit their nests occurs within 24 hours (Evans & Freeman, 1950) or several days (Hartwell et al., 1958) but there are few other observations on the mechanism of infestation. Investigations on bird fleas (Bates, 1962; Humphries, 1968) show highly evolved adaptations for host recognition.

The exchange of fleas between hosts was studied on the rabbit flea, *Spilopsyllus cuniculi* by Mead-Briggs (1964) who found that there was a considerable exchange of fleas between rabbits already after 3 days of infestation. Williams (1971) found that “two rabbits upon which fleas had been released had lost 94 and 96% of their fleas within 10 days”. Indeed, the rabbit fleas seem to spend most of their time “away from the host in the rabbit burrows” (Williams & Parker, 1971) or in the field. As was shown by Mead-Briggs (1964) rabbits are able to pick up 45% of rabbit fleas scattered free at low densities in a pasture within 25 days. This is possible because of the attraction of the fleas to the urination places of the rabbits (Vaughan & Mead-Briggs, 1970). A similar behaviour may account for the extensive exchange of fleas in hedgehog populations, although not shown by Sgonina (1935) who investigated the reaction of the hedgehog fleas to various stimuli emitted by its host.

ACKNOWLEDGEMENTS

The field work was done at the Ecological Station, Ölands Skogsby, Sweden. We express our sincere thanks to Professor Bertil Kullenberg, head of the station. We are also greatly indebted to Mr Michael Moon, B. Sc., for the linguistic revision.

REFERENCES


Allanson, M. 1934. The reproductive process of certain mammals. VII. Seasonal variation in the reproductive organs of the male hedgehog. Philos. Trans., London (B) 223, 277–303.


Löfgqvist, J. 1973a. Seasonal weight changes of the hedgehog, Erinaceus europaeus L. In manuscript.


— 1973c. Home ranges and migration in the hedgehog, Erinaceus europaeus L. In manuscript.


Rothschild, M. & Ford, B. 1964a. Breeding of the rabbit flea (Spilopsyllus cuniculi (Dale)) controlled by the reproductive hormones of the host. Nature 201, 103–104.

— 1964b. Maturation and egg-laying of the rabbit flea (Spilopsyllus cuniculi Dale) induced by the external application of hydrocortisone. Nature 203, 210–211.


On Flight Thresholds for Temperature and Wind Velocity, 24-hour Flight Periodicity and Migration of the Water Beetle Helophorus brevipalpis Bedel (Coleoptera, Hydrophilidae)
most ready fliers among water insects, always equipped with well-developed wings and flight muscles. It has other characteristics typical of migratory insects (Landin, 1968). However, it is small and slow and must be considered as a weak flyer. Its flight periodicity during the 24-hour period and its flight threshold for temperature and wind velocity has been studied by Landin (op. cit.) at a lake (Sågsjön, near Stockholm) which is permanent when compared with the pools on the Alvar. The lake is surrounded by woodland and is fairly well protected from the wind. The present paper aims to extend Landin's results and to compare them with those obtained during a similar investigation at the ephemeral waters on the Alvar, which are subjected to a different climate (e.g. much more exposed to wind), and which have a different physiognomy and surroundings. The results are also discussed in the light of general theories on insect migration.

THE LOCALITY

The investigation took place at the Ecological Station of Uppsala University, Ölands Skogsby. It is situated at the edge of the Alvar.

The locality was originally a pasture, and areas to the east and south are still grazed. Close to the station there is an ephemeral water body on lime-stone flags which are covered by soil. Near the northern shore is a line of planted willows; there are no other trees in the vicinity.

The size of the pond varies greatly (Table 1). It may be completely dried out already in August, or it may last into October.

The pond (see Fig. 1) is used as a source of water by cattle and is polluted by faeces and urine. Accordingly, it is very eutrophic and productive. The dominating macroscopic plants are Carex probably disticha Huds., Scirpus uniglumis Link and Phleum phleoides Karst. (plants determined by Mr Eje Rosén, University of Uppsala). The part to the north of the electric cable contains less vegetation than the southern part. The northern part is deepest, its

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Table 1. Maximal "length" (SSW–NNE) and maximal "breadth" (ENE–WSW) in metres of the ephemeral pond at the Ecological Station

An excavated pit at the southern end is excluded. All figures are in metres and are from 1965. On March 20 the pond was partly frozen

<table>
<thead>
<tr>
<th>Date</th>
<th>Length</th>
<th>Breadth</th>
</tr>
</thead>
<tbody>
<tr>
<td>March 20</td>
<td>100</td>
<td>45</td>
</tr>
<tr>
<td>June 16</td>
<td>95</td>
<td>32</td>
</tr>
<tr>
<td>July 30</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>September 30</td>
<td>13</td>
<td>17</td>
</tr>
</tbody>
</table>
Table 2. Number of H. brevipalpis trapped during 34 days by glass traps emptied each hour

<table>
<thead>
<tr>
<th>Time of year: May to June</th>
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</thead>
<tbody>
<tr>
<td>Date</td>
</tr>
<tr>
<td>Number of specimens</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Time of year: July to August</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date</td>
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<tr>
<td>Number of specimens</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time of year: September</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date</td>
</tr>
<tr>
<td>Number of specimens</td>
</tr>
</tbody>
</table>

The maximal depth being approximately 1 m. This is the last part to dry up and is frequently used by cattle. Cows trample the shore and bottom, making the habitat still more unstable.

The pond fauna is very rich. When stirring the soil along the water's edge one can see that the surface sometimes is literally alive with beetles belonging to the palpicorn genera Ochthebius, Limnebius, and Helophorus.

The investigation took place in 1965, 1966 and 1967, and the sketch map was drawn in 1969, but in the mean time the pond and its surroundings did not alter.

Each of the four traps consisted of a pane of window glass 66 × 102 cm arranged in a wooden frame lying on the ground. The pane sloped with the shorter and lower side over a water trough (see Fernando, 1958 for further details). The sites of the traps are shown in Fig. 1. The greenhouse indicated was completed April 1, 1967.

This type of trap attracts water insects by light reflection from its glass surface and most of the

<table>
<thead>
<tr>
<th>Table 3. Mean monthly precipitation in mm at Mörbylånga, Öland during the period 1931 to 1960</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan.</td>
</tr>
<tr>
<td>44</td>
</tr>
<tr>
<td>62</td>
</tr>
</tbody>
</table>

MATERIALS AND METHODS

The materials consist of Helophorus brevipalpis captured in flight during different periods in 1965, 1966, and 1967 (see Table 2). Most of them are believed to come from the pond described above, although other waters must have contributed to the catch.
beetles gather in the trough. Landin (1968) used such traps and discussed the function of them.

The traps were emptied every hour as long as water insects flew into them. The weather conditions were also noted hourly. A total of 20,117 H. brevipalpis were caught during 34 days. They are grouped day by day in Table 2.

In 1965 and 1966 a Pennsylvania light trap (Frost, 1957) was used to catch water insects at hours when the glass traps had ceased to catch them (see also Landin, op. cit.). In July 1967 a Rothamsted light trap was used. The light trap was operated almost every night during the periods when the glass traps were used, however no light trap was used in May 1967. The light traps were operated from before sunset and for a total of 74 nights.

The following weather parameters were measured:

(a) air temperature by a Fuess thermograph and a Hg-thermometer to correct the thermograph, both being at 1.5 m height in the screens shown in Fig. 1.

(b) relative humidity by a Fuess hygrograph also placed in the screens, both recorders being set for weekly operations.

(c) wind velocity by a Fuess cup counter anemometer (Fig. 1) placed at 1.5 m height, measuring the total wind run passing during one hour, from which the mean wind velocity was calculated.

(d) the illuminance by two photometers, Gossen Trilux and UVA-lux, directed towards the sun or the lightest part of the sky.

The amount of cloud was estimated by means of a scale of nine degrees. The time is Middle European Time.

RESULTS

The glass traps naturally capture those water insects which fly near the ground and which actively orientate towards and alight on them. When placed in the vicinity of water, as here and at Lake Sågsjön, we believe that they catch insects that have just started their flight. After having observed flying H. brevipalpis at the nearby pond it was estimated that a specimen needs no more than 2 or 3 minutes to reach trap no. 1 (Fig. 1). It was also frequently observed that the traps catch specimens of H. brevipalpis when the species is flying at the pond. Furthermore, specimens taking off from the shore often only fly some few meters before re-alighting on the shore. Our catches probably consist of both emigrants from and immigrants to the nearby water body (see also Landin, 1968).

In 1965 not a single H. brevipalpis was caught in the light trap. In 1966 we captured 22 specimens on four evenings in July and August. 8 of them on July 17 before 22.00. On two evenings out of twelve in 1967 the species was trapped with the light trap, but in small numbers compared with the glass trap catches. One of these evenings was on July 7, when the work had to be discontinued one or two hours before the glass traps ceased to catch H. brevipalpis.

We reached the conclusion that the species mostly flies during daytime and that the catches given by the glass traps are good indicators of the time of flight and the numbers flying. This is in agreement with Landin (op. cit.).

Attempts were made to detect a general pattern in the flight periodicity by summing all catches from different days, hour by hour, and calculating the mean (but only from those days when H. brevipalpis flew, indicated by catches). We distinguish between May to June, July to August and September. The result is given in Fig. 2. July and August 1965 are also distinguished from the same months in 1966 and 1967. It should be observed that the catch on August 3 and 4, 1965, constitute 90.5% of the total catch from July and August that year. The weather on those two days was very unsettled. The catches before and after those two days are small and uniform (Table 2). We have not calculated the mean for this period but give the actual figures, which are discussed below.

From Fig. 2b it is obvious that the catch from Lake Sågsjön consists of two peaks. When plotting the cumulative catch hour by hour on probability paper (see Lewis and Taylor, 1967, pp. 50 ff.) it almost

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Fig. 2. Glass trap catches of H. brevipalpis over 24-hour periods at different times of the year from 1965 to 1967 at Ölands Skogsbry and in July, 1965 and 1966, at Lake Sågsjön. The figures in a, b, d, and e are means calculated from the days when catches occurred (see Table 2). (c) The total numbers; the shaded part shows the catches, excluding those of August 3 and 4, 1965. On August 3, 16.00–17.00 hours, the catch in trap no. 4 was lost, while the whole catch for 17.00–18.00 hours was lost (the field notes state ‘masses’). On July 7, 1967, the work was discontinued early which is why the last bar in e is broken. In Fig. 2a the actual numbers between 11 and 21 hours varied from 1 to 7 specimens. The means are all stated as 1. The curves are of cumulative probability and refer to the scales on the right of each diagram.
Fig. 2
perfectly fits a sigmoid curve, i.e. it forms two peaks. Also, they are skewed away from noon. A hint of two peaks appears in the material from Ölands Skogsby during July and August 1965. When plotting it we excluded the hour 17.00–18.00, but not the hour 16.00–17.00 (see Fig. 2). If the peak between 13.00 and 14.00 had not existed this material would also have consisted of a morning and an afternoon peak.

The material from Ölands Skogsby in July and August, 1966 and 1967, also consists of two peaks and generates a sigmoid curve, but the catches are not so sharply divided in a morning and an evening peak as at Lake Sägsjön. The missing catch after 20.00 hours on July 7, 1967 only slightly influences this result.

During the period May to June, 583 (or 96.6%) of the total catch of 603 specimens are from only two days, May 22, 1967 and June 23, 1965. Almost all of them were caught in the mornings (Fig. 2a).

During the afternoons a total of only 26 beetles were caught but according to the probability curve they may indicate an afternoon peak.

During September there is no peak in the catch. The flight was restricted in time during the day compared with the other periods.

On days when the glass traps were not emptied once an hour they were emptied after they had ceased to catch water insects. Such day-total catches must not be compared to catches summed hour by hour. It was found that in spite of detergents and straw in the water troughs the beetles were able to leave the traps and more so when they are left unattended.

Catches accounted for in Table 2 from the same times in different years are of about the same sizes (compare August 1965 to August 1966 and the catch on July 17, 1966 to July 3, 5, and 7, 1967). When compared with each other day-total catches from the same times in different years are also of about the same size. To judge from the day-total catches before and after July 17, 1966, a large migration began on that day, or perhaps July 16, and possibly continued during the whole of late July, as we captured 774 H. brevipalpis on August 3 and 4, 1966.

We have analysed the catch in 1965 as regards the dependence on temperature and wind velocity by calculating the flight occurrence (catch frequency or occurrence) according to Taylor (1963). The hours during which the glass traps were operated were grouped into classes comprising 1°C and 0.5 m/s. In accordance with Landin (1968) the minimum temperature each hour was used when deciding in which class the hour should be placed. The reason for this was that some beetles might arrive at the traps in straight flight at temperatures too low for a new take-off. Accordingly, the minimum temperature should decide if beetles were flying or not. The night hours 22.00–03.00 in June, 21.00–04.00 in July and August and 19.00–05.00 in September were excluded as low illuminance might have constituted a threshold (see Landin, op. cit.).

The catch occurrence was then plotted against temperature and wind velocity (see Figs. 3 and 4). A temperature threshold for flight was found and its mean was placed at 12°C. The hours with no catches above 19°C are probably of little importance, as they are only 11 out of a total of 385 hours. The number of hours below 12°C is 58. By using the maximum temperature we also get a threshold at about 12°C. When searching for a threshold for wind velocity we excluded (inter alia) hours with temperatures below 12°C. In the 301 hours remaining there is a clear relationship between catch frequency and wind velocity, but no clear threshold. The air temperature
and wind velocity were positively correlated in the material from Lake Sågsjön (r: 0.762; f: 50, p < 0.001) but did not seem to be correlated during four randomly selected days at Ölands Skogstby, (one from May–June, two from July–August and one from September) (r: 0.144; f: 52, not significant).

In Table 4 we have pooled these temperature intervals during which the first catch occurred each day in 1966 and 1967. On 6 of these 10 days the first catch occurred at 12°C.

**DISCUSSION**

The numbers of beetles trapped per day during May–June and September are very small compared with those in July and August (Table 2 and Fig. 2). The species passes different phases of the life cycle during these periods. In England *H. brevipalpis* emerges from the pupa predominantly in June–August and is reported to fly and invade waters mostly from mid June until mid August (Fernando, 1958, pp. 132–37). These adults hibernate and the following spring far fewer are to be trapped flying. Fernando found larvae during June and until late July, indicating the new generation. Our results are in agreement with these statements. The small amounts trapped during May and June were old and overwintered specimens, hard, and dark coloured. During July and August most were young, i.e. callow and more light-coloured, while in September the trapped beetles seemed to be harder and darker again. So, obviously the large numbers flying in the middle of summer are young beetles migrating to new breeding or hibernation places. This is in accordance with Johnson's theories (1960, 1969 pp. 36 ff. etc.). *H. brevipalpis* also seems to belong to Johnson's migration class IIIa (Johnson, 1965, p. 191, 1969, pp. 29 ff.).

It is not known whether *H. brevipalpis* makes non-migratory flights in the ontogenetical sense (see Johnson 1963, 1969, pp. 213 ff.) but we suppose it does not. According to Southwood (1962, p. 190) the great majority of flights of Coleoptera, especially aquatic beetles, are migratory. He meant migration in the ecological sense, i.e. leaving the habitat. However, as pointed out on p. 108 many *H. brevipalpis* specimens make short flights at the habitat and consequently this species seems to be an exception to the general rule for aquatic beetles. The migration of *H. brevipalpis* specimens may very well consist of several flights. In Britain the first eggs are laid between November and January (Fernando, op. cit.) but in Sweden this probably does not occur until spring. This gives ample time for migration in the ontogenetical sense (Johnson, op. cit.). Fernando (op. cit. pp. 124–26) found that *H. brevipalpis* probably selects habitats by arriving indiscriminately at them and by leaving unsuitable ones. Pajunen & Jansson (1969, p. 412) concluded that rock pool corixids probably disperse in June by short flights and slowly expand the area occupied. Perhaps *H. brevipalpis* behaves similarly. The problems facing these different species must be much the same, since they occupy similar habitats consisting of numerous small, temporary pools.

However, a small part of the flying population probably joins the aerial plankton. Hardy & Milne (1938, p. 227) caught one specimen of *H. brevipalpis* 250–300 feet high in the air. Glick (1939, p. 30) captured one unidentified *Helophorus* specimen at 200 feet. *Helophorus* spp. is an item in the food of the swift (Lack & Owen, 1956) which may also indicate dispersal high in the air. The records of *H. minusus* F. by Freeman (1945, p. 137) and *H. glacialis* Villa by Östbye (1969) also suggest that this type of beetle may become wind-borne.

Pajunen & Jansson (op. cit.) observed an increased tendency in rock pool corixids to fly at times of major habitat changes. Major habitat changes are the thawing in spring and the refilling of the pools, by increased precipitation, from July onwards. The situation is similar for *H. brevipalpis* on Öland. The flights of hibernated adults in May (Table 2, Fig. 2) take them to large numbers of ponds refilled by the thaw and maintained because of lower evaporation. When the precipitation increases in July vast numbers of callow adults fly. The rainfalls then retard the drying up of the ponds and perhaps more habitats remain available. Table 3 states the mean monthly precipitation at Möbylänga and it is about the same at three other meteorological stations on Öland. At Stockholm and Lake Sågsjön the sequence of mean monthly precipitation is similar.

The bimodal pattern of flight is probably the normal in Sweden and in Britain (see Lewis & Taylor, 1965, pp. 425–26, 447) during the middle of the summer. The causes of this pattern are unknown but it appears that temperature determines the initiation of the flights in the morning (see Table 4). This is generally the case in most day-flying species (Johnson, 1965, p. 196). The illuminance was always...
Table 4. Maximum and minimum temperatures and illuminance in lux at first catches and immediately after last catches of H. brevipalpis at Ölands Skogsby

On July 7, 1967 the precise time at the last catch is unknown (see p. 108). In July 1967 we did not measure illuminance after the last catch.

<table>
<thead>
<tr>
<th>Month</th>
<th>Date</th>
<th>Hour</th>
<th>Temperature intervals °C</th>
<th>Illuminance in lux × 10⁵</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>First</td>
<td>Last</td>
<td>First</td>
</tr>
<tr>
<td>1966</td>
<td>July</td>
<td>17</td>
<td>05-06</td>
<td>20-21</td>
</tr>
<tr>
<td></td>
<td>August</td>
<td>3</td>
<td>06-07</td>
<td>18-19</td>
</tr>
<tr>
<td>June</td>
<td>August</td>
<td>4</td>
<td>06-06</td>
<td>20-21</td>
</tr>
<tr>
<td></td>
<td>August</td>
<td>9</td>
<td>04-05</td>
<td>19-20</td>
</tr>
<tr>
<td>1967</td>
<td>May</td>
<td>22</td>
<td>06-07</td>
<td>18-19</td>
</tr>
<tr>
<td></td>
<td>May</td>
<td>23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11-12</td>
<td>16-17</td>
</tr>
<tr>
<td></td>
<td>May</td>
<td>25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>07-08</td>
<td>16-17</td>
</tr>
<tr>
<td>June</td>
<td>July</td>
<td>3</td>
<td>05-06</td>
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<tr>
<td></td>
<td>July</td>
<td>7</td>
<td>04-05</td>
<td>?</td>
</tr>
</tbody>
</table>

<sup>a</sup> Only two specimens captured this date.
<sup>b</sup> Only five specimens captured this date.

much higher than a possible light threshold at the time of our first catches. In the evenings, when H. brevipalpis ceased flying, temperatures and illuminance were not limiting factors at Lake Sågsjön (Landin, 1968). Neither was the illuminance at Ölands Skogsby limiting for a beetle capable of flying until midnight. However, the temperatures may possibly have interrupted the flight as they were only slightly above 12°C on 8 of the 10 days in Table 4.

This difference is certainly due to different physiognomies of the two habitats. Lake Sågsjön is surrounded by heights and woody grounds and protected from winds. Its shores are a much more closed habitat than the Alvar of Öland and must retain the heat more effectively and longer during the day. Temperature measurements indicate that the air temperature at Lake Sågsjön is high, i.e. above 14 or 15°C, later in the evening during July and on several occasions it was well above 12°C during the whole night. In spite of this the light trap only seldom captured H. brevipalpis, although regularly operated from spring until autumn together with glass traps in 1966 and part of this period in 1965.

At Lake Sågsjön, Landin (op. cit.) did not detect any connection between wind velocity and catch frequency. The temperature threshold in the material from this locality was between 11-15°C, an interval which covered the threshold in the material from Ölands Skogsby (12°C). The latter should be much better defined as the sample is much larger.

As we do not know the body temperature of the beetles we should perhaps consider this temperature threshold as only apparent. We do know that it is a threshold for flight as we measured the temperature at a height of 1.5 m and trapped the beetles away from the take-off sites (see Cockbain, 1961; Lewis, 1963). However, the body temperature of small insects such as these certainly do not diverge from the air temperature and when leaving or arriving at the habitats the great majority fly higher than 1.5 m. From an ecological point of view a flight threshold may be more interesting than a threshold for take-off. Laboratory experiments (Landin unpubl.) indicate a threshold for take-off between 12 and 15°C.

Fig. 4 indicates the importance of wind velocity for the catch frequency. Compared with the rest of July and August 1965, August 3 and 4 were calm;
the mean wind velocity being 4 m/s or more during only five of the 33 hours. In contrast, 57 hours out of 116 from the remainder of the period had a mean wind velocity of 4 m/s or more. The catches then were small compared to both August 3 and 4 and July–August 1966 and 1967. During only 17 out of a total of 116 hours from the latter period was the mean wind velocity 4 m/s or more. The catches from this period were uniform and large. This further indicates the importance of wind velocity.

The weather obviously influences the seemingly usual bimodal pattern of flight in summer. On July 3, 1967, we recorded our most extreme bimodal pattern. It was more extreme than in Fig. 2e and with a much lower minimum during the middle of the day. The weather was settled and similar to that of the July days at Sågsjön (see Landin, 1968). The insolation and temperature changed evenly, with a maximum at noon and at 14.00 respectively. The wind velocity also had its maximum during the middle of the day, while the equally regular RH curve had its minimum. With the exception of the evening, the amounts of cloud were small. All these parameters behaved irregularly on August 3 and 4, 1965, when we recorded our most disturbed pattern, as may be inferred from Fig. 2c; the morning peak belonging to August 4, the others to August 3. The weather was unsettled and showers occurred. The three peaks in the catch were associated with changes in weather factors summarized in Table 5. From this and other occasions, both at Sågsjön and at Ölands Skogsbry, we consider that periods of sun and calm after rain on summer days usually encourage large flights. Probably the bimodal pattern is more often disturbed by weather in an open habitat such as the Alvar than in a closed one as at Sågsjön. The pattern in Fig. 2e is not as regular as in 2b. This may depend on weather factors behaving more regularly in a sheltered habitat. The temperature and wind velocity, for example, were correlated at Sågsjön but not at Ölands Skogsbry.

It is not known how the wind velocity affects the beetles. *H. brevipalpis* lives at the water surface—if there is sufficient aquatic vegetation—at the water’s edge or in water only a few centimetres deep. The species cannot swim (Angus, 1966). It moves, and even forages, on the shore and takes off from there. Even small waves on puddles and ponds may interfere with the beetles leaving the water (according to observations by Landin) and consequently may decrease the numbers taking-off. Palmén (1955, p. 27) thought that chironomids gain by emerging during night when wind velocity and waves are less.

Wind velocity may be one factor, among others, directly interfering with the take-off. It may stop take-off or cause the beetles to fly only locally, perhaps only some metres in the vicinity of the habitat. Such flights do, in fact, occur. Our traps were not able to indicate them but caught only beetles that actually left the habitat. Young (1965, p. 227) discusses the significance of the wind velocity further, concerning the initiation of flight in corixids.

The morning and the evening peaks in our catches imply that the beetles mostly fly during comparatively low wind velocities. The velocities at Lake Sågsjön and at Ölands Skogsbry are usually lower during mornings and evenings, and this is probably general. Another factor is that convection is seldom maximal during morning and evening (see e.g. Lewis & Taylor, 1965, p. 409, Fig. 11).

The bimodal flight pattern may be an evolutionary compromise between the need for an undisturbed take-off and the probable value of being wind-borne and widely dispersed. Lewis and Taylor (op. cit. pp. 409–14) state that day-flying insects are more disposed to get wind-borne than night-flying ones. Johnson (1969, p. 276) points out that crepuscular flying insects tend to be less disposed to regular wind-borne transport. Accordingly, from the point of view of dispersal, it should be most advantageous to restrict the flight period to the middle of the day, with a unimodal pattern of flight.

The bimodal pattern may have another advantage. When invading new water bodies the beetles need to direct the last part of their flight course. Temporary water bodies are discontinuous, often numerous, especially on the Alvar, but scattered and often occupying only small areas. Low wind velocities should be advantageous in this situation. According to the data of Bruce (1964), and our own experiences, *H. brevipalpis* together with *H. minutus* Fabr. are the two water beetles most characteristic of the Alvar. The great majority of water insects invading the small, artificial habitats set up by Fernando (op. cit. Tables 2 and 3) was *H. brevipalpis*. See also Grensted (1939). The first of the present authors has found the species in numbers in very ephemeral rain water puddles in the vicinity of Lake Sågsjön, and it is common in temporary pools in gravel-pits. Therefore we consider that *H. brevipalpis* belongs to small, temporary water bodies, and we think the bimodal
pattern of flight may be an adaptation to its habitat, facilitating its colonization.

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REFERENCES


Seed-bugs (Het., Lygaeidae) from the Great Alvar of Öland, Southern Sweden

Some observations on their distribution in relation to food and microclimate

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ABSTRACT

Coulianos, C.-C. (Department of Zoology, University of Stockholm, Stockholm, Sweden.) Seed-bugs (Het., Lygaeidae) from the Great Alvar of Öland, Southern Sweden, with some observations on their distribution in relation to food and microclimate. ZOON, Suppl. 1: 115-122, 1973

In different habitats of the Alvar (a steppe-like formation type on Ordovician limestone bedrock), 28 spp were recorded. 19 spp. occurred regularly in breeding populations, 15 of which were considered a characteristic part of the Alvar fauna. With the exception of the possibly endemic Planosoma trichopterus (Thoms.), and the Carex-inhabiting Cymus glandicola Hahn and Pachybrachia fracticollis (Schill.), most species are much more abundant in habitats outside the Alvar area, esp. in sandy areas. This may be due to larger food supplies (seeds) being available in such habitats. Microclimatic recordings during the summer showed distinct differences between lygaeid habitats. Within the sunny, dry, and windy climate of the Alvar, microhabitats exist which permit the permanent existence of species demanding more stable and humid conditions. The extremely heterogeneous distribution of such habitats, however, may not permit the existence of lygaeids with high demands on stable, microclimatic conditions. Such species, normally occurring in habitats with thick litter layers, e.g. most woodland species, are all lacking on the Alvar. Over large areas, the drainage is very poor. Such areas are often submerged during the wet season. Here the lygaeid fauna is extremely poor, sometimes consisting only of an adult population of Nysius thyri (Wilfr.) during the dry season.

INTRODUCTION

On the island of Öland, situated in the southern Baltic, about 25% of the land area consists of so-called Alvar ground. This is a steppe-like formation type on Ordovician limestone bedrock. The main part of southern Öland consists of Alvar ground. Here the steppe-like waste extends for 45 km in a N-S direction and with a maximum breadth of 10 km. This is the “Great Alvar”. In addition, smaller Alvar areas are found in the central and northern parts of the island.

The flora and vegetation has been much studied and its main characteristics are well known (Sterner, 1938, 1950; Albertson, 1950). The Alvar vegetation is mainly the result of combined actions of the limestone bedrock, climatic factors and the cultural influence of man and grazing animals. For large parts of the Alvar, the shallow soil-layer, the continuous winds and the strong insolation seem to be very important from an ecological point of view.

Unfortunately, there is no meteorological station representative for the Great Alvar, and many of its bioclimatic aspects are not well understood. At the Ecological Station of Uppsala University, which is situated on the edge of the Great Alvar, meteorological records have been kept since July 1964, and when worked through, these will give most valuable information of the Alvar climate.

As a whole, the climate of Öland is maritime. The precipitation is rather low. An estimate with the aid of records from different meteorological stations on Öland, gives a figure of about 400 mm per year for the Great Alvar. Important from an ecological point of view, is the irregular distribution and magnitude of precipitation during the driest period of the year.

Over large areas of the Alvar, a very shallow soil-layer rests on the level flags of rock, and as a consequence, there is little or no drainage. Such grounds usually dry up quickly during dry summer weather, even after heavy rains. This is due to the strong insolation, which in combination with dry air and strong winds, results in high evaporation during the summer months. During other seasons, when the evaporation is slight, the same grounds may be waterlogged or even submerged (cf. Bergsten, 1955). This is considered to be one of the most important conditions
influencing the distribution of plants and plant communities on the Alvar (Sterner, 1950), and applies no doubt to many members of the fauna as well.

Climatic conditions of a more immediate ecological interest, i.e. the microclimates of the Alvar, have been very little studied. Falck (1913) gives some information on air and ground temperatures during July, 1912, and Wahlgren (1915) records some isolated measurements. The fauna of the Alvar areas of Öland has been studied by Wahlgren, (1915, 1917), who gave a valuable and comprehensive survey of most animal groups, including 14 species of Lygaeidae. Further records were given by Jansson (1929).

Most of the Swedish lygaeids are ground-living insects, living in rather dry, sun-exposed habitats, feeding on fallen seeds (Coulianos, unpubl.). With regard to the characteristic vegetational and climatic features of the Great Alvar, a study of its lygaeid fauna should elicit valuable information for an understanding of the ecology of these insects.

The purpose of this paper, which is to be seen as a report of work in progress, is to give a list of the lygaeid species so far found on the Great Alvar, and examples of microclimatic conditions in some principal lygaeid habitats.

**LYGAEID SPECIES FOUND ON THE GREAT ALVAR**

The records are all made by the present author (unless otherwise stated), and is the result of collecting during most months of the year. Species which are considered to be characteristic of the fauna of the Alvar are marked with an asterisk.

*Lygaeus equestris* (L.). Common, especially in the northern part. Mostly associated with *Cynanchum vincetoxicum* (L.) Pers., where the fallen seeds seem to constitute the principal food for larval instars I–III. Older larvae and adults also feed on seeds of many other plants, e.g. *Centauraea scabiosa* L., *Cirsium vulgare* (Savi) Ten. and *Taraxacum* spp.

*Nysius thymi* (Wlf.). Very common on the whole area, and in most habitats. Especially characteristic of grass steppe formations with, e.g., *Festuca ovina* L. and *Agrostis* spp., together with a variety of low-growing plants. Also on moraine ridges dominated by *Arrhenatherum elatius* (L.) J. et C. Presl. In such habitats it is often very abundant. It is one of the few species that are found in abundance in areas which often are submerged during the wet season (see above). During the dry season, adults may occur in high numbers, feeding in such areas. The larval instars I–III keep mostly to the ground, feeding on fallen seeds. Later instars and adults are often found on plants and grasses, feeding upon their seeds.

*Nysius ericeae* (Schill.). Only found in the southern part, E. of Segerstad, 3.VIII.1961 under *Caltha palustris* (L.) Spreng. This species has been confused with *N. thymi*, and earlier records should be revised. However, it is evident that *N. thymi* is the much more common species on Alvar grounds.

*Ortholomus punctipennis* (H.-S.). Occurring sparsely. A few records only, especially along S.-exposed parts of stone-fences, and always associated with *Potentilla* spp. This species is more common in sandy habitats in other parts of Öland.

*Cymus glandicolar* Hahn. Very common and often abundant on Carex spp. over the whole area. Found on *Carex elata* All., *C. panicea* L., *C. oederi* Regel., *C. nigra* (L.) Reich., and *C. hostiana* DC. Very characteristic of the vegetation associated with the numerous temporary water pools. All stages are associated with the spikes of the plants. On the Alvar it occurs in the same abundance as in habitats outside the Alvar area.

*Geocoris clypeatus* (L.). Very common and sometimes abundant, although with a very heterogeneous distribution. Characteristic of the so-called Karst-Alvar, where the limestone rock flats have a thin, patchy vegetation with lichens, mosses and low-growing plants in crevices in the rock forming vegetation "strings" (Fig. 1). It is a facultative predator, which has been observed to feed on eggs and small larvae of Lepidoptera, Heteroptera and Heteroptera e.g. *Chlamydomus evanescens* Boh. It also feeds upon seeds.


*Macroplex preysleri* (Fieb.). Common over the whole area and associated exclusively with *Helianthemum* spp. Found on *H. oelandicum* (L.) DC and *H. nummularium* (L.) Mill. Larvae and adults feed on the seeds, and are mostly found in the seed capsules lying on the ground, distributed in a characteristic pattern around the plant. This applies especially to the smaller larvae. It also feeds upon the plant. Wahlgren (1915) recorded *Oxycarenus modestus* Fall. from the Great Alvar, but as has been shown by Coulianos (1973), this refers to *M. preysler-
lepi. This species seems to differ greatly in abundance from year to year.

*Pachyrhachius fracticollis* (Schill.). Rather common, but seldom abundant, although it attains the same abundance on the Alvar as in habitats outside this area. In the same habitats as *Cynus glandicolar*, but only found on *Carex-spp*, growing in denser clumps, e.g. *C. elata*, where it mostly keeps to the lower parts of the plant. In spring, however, it often occurs high up in the vegetation, flying at that time.

*Ligyracris silvestris* (L.). Rather common, but only found on more or less moist Alvar-meadow, often in the vicinity of water pools, especially where *Potentilla fruticosa* L. is dominant. Larvae and adults have frequently been observed feeding on the seeds of this plant, but also on seeds of many other plants. It occurs in the same type of habitat in other Alvar areas on Öland, but outside such areas it is very rare on Öland (3 records only).

*Megalonota praetexta* (H.-S.). Only found twice on the edge of the Alvar. Rather common in sandy areas in other parts of Öland, where it has been found associated with *Geranium molle* L. and *Erodium cicutarium* (L.) L'Hér.

*Fig. 1. Öland, Resmo Alvar. Part of the Karst-Alvar, where the microclimatic measurements were made. Photo: Jan Landin. June 10, 1963.*

*Megalonota chitra* (F.). Common in dry, sun-exposed habitats, characterized by a variety of low-growing plants. Especially abundant along S.-exposed stone-fences.

*Tropistethus holosericeus* (Scholtz). This small species is easily overlooked, but seems to be rather common. It is mostly found under flat pieces of limestone, lying on a thin layer of black earth. Along the edges of such stones, fallen, wind-blown seeds accumulate and the bugs have been observed transporting such seeds into their hiding places under the stones. It is also found in moss growing in cracks in the bedrock.

*Ischnacris angustifolius* (Boh.). Very common and abundant almost everywhere on the drier part of the Alvar. It is especially abundant along the vegetation strings of the Karst-Alvar, and amongst mosses and lichens on the flat bedrock.

*Pionosoma trichopterum* (Thoms.). This species was originally described from the Borgholm Alvar in 1870, and has so far only been found on Öland, and always on Alvar ground. It seems to be very local, but occurs sometimes in abundance, e.g. on the Lenstad Alvar. All specimens found agree fairly well
with the original description, but as several species have been recorded from isolated areas in SE.
Europe only, and as a modern revision of the species has not been made, the status of this species is un-
certain. Whether it is a true endemic species, or a
SE-European species with a disjunct distribution,
has yet to be investigated. However, it is fairly
distinct from *P. varius* (Will.), which does not occur
on Alvar ground, but is common in sandy habitats
with exposed sand in most parts of Öland.

*Plisthisus pusillus* (Scholtz). This small species is
easily overlooked, but is very common, especially
under mosses, growing on the limestone bedrock
along vegetation strings on the Karst-Alvar, and
under stones, where it transports fallen seeds.

*Acomnus rupestris* (Will.). Found only once, on
Resmo Alvar 25.VII.1963, as larvae on the flowers
of *Valeriana dioica* L.

*Stygnoconis sabulosus* (Schill.) (pedestris (Fall.)).
Common in most parts of the area. Always in shaded
habitats with high relative humidity, e.g. in thicker
moss, under dense vegetation, in moss at the base of
Juniperus- and Potentilla-shrubs, and along the N.-
exposed side of stone-fences. It often occurs abund-
antly.

*Stygnoconis fuliginosus* (Geoffr.). Occasionally
found, and only on the grass-steppe-Alvar. No larvae
have been found.

*Perithecus nabilus* (Fall.). Found in most parts of
the Alvar. Always on dry ground, often along the
S.-exposed side of stone-fences. It never occurs in
abundance on the Alvar, which is in sharp contrast
to its occurrence in sandy habitats, especially at the
edges of cultivated grounds, where it can be extremely
abundant.

*Trappezonotus arenarius* (L.). Very common and
mostly abundant everywhere, but always on dry
ground. Together with *N. thymi* and *I. angustulus*,
this is the most common lygaeid species on the Great
Alvar. Like the foregoing species, it is much more
abundant in sandy habitats, especially at the edges
of arable land.

*Sphragisticus nebulosus* (Fall.). Occasionally found
at the border of the Alvar. Like the foregoing species,
it can be extremely abundant at the edges of arable
land.

*Aphanus rolandi* (L.). Only two records from
the Great Alvar: E. of Resmo, 26.VIII.1939, 1 ex. (H.
Lohmander leg.) and Resmo Alvar, 23.VI.1970,
4 exx.

*Rhynarochromus pini* (L.). Occasionally found in
habitats with more varied vegetation, especially
along S.-exposed stone-fences. It never occurs in
greater numbers on the Alvar. Like several other
species, it is much more common in many other
habitats on Öland, e.g. sandy heaths or at the edge
of arable land, where it can be extremely abundant.

*Gropoteslymnes* (F.). Occasionally found at
the edge of the Alvar and always associated with
Boraginaceae, e.g. *Cynoglossum officinale* L. and
Anchusa officinalis L.

*Emblethis verbasci* (F.). Occasionally found in dry,
sun-exposed habitats, e.g. along S.-exposed stone-
fences, with a wide variety of low-growing plants. In
such places it has sometimes been found in abun-
dance. The presence of flat pieces of stones, especially
limestone, seems essential for the occurrence of this
species on the Great Alvar. Under such stones, dense
aggregations of larvae and adults can be found. It is
essentially a species of dry, sandy habitats, where it
can be very abundant.

*Gonianotus marginipunctatus* (Will.). Rather com-
mon in dry, sun-exposed habitats over the whole
area and particularly on the Karst-Alvar. This spe-
cies is also characteristic of sandy habitats, especially
sandy grass-heaths, where it is often very abundant.
Although it never occurs in great abundance, it
seems to be a very characteristic member of the
Alvar fauna.

*Taphropelus contractus* (H.-S.). Occurring sparsely
on the Alvar. It has only been found along S.-
exposed sides of stone-fences, where it occurs in moss
among low-growing plants, feeding upon their seeds.
This species has been confused with *T. hamulatus*
(Thoms.). However, all specimens recorded from
Öland are *contractus* (Coulianos, 1973).

SOME BIOCLIMATIC OBSERVATIONS
IN LYGAEID HABITATS

With regard to the often very characteristic, hetero-
genous distribution of most species, mentioned
above, and with regard also to the fact that most of
the species found on the Great Alvar are ground-
living, occurring in habitats where great differences
in microclimatic conditions can be expected, it
seemed of interest to describe such habitats in micro-
climatic terms.

During shorter periods in different seasons, record-
ings have been made of the microclimate of some
principal lygaeid habitats. The examples given here
were all recorded within an area of the Karst-Alvar, 400 m. ESE. the southern point of the Alvar "lake" Mőckalmossen. The vegetation is varied, and bare patches of limestone bedrock alternate with areas thinly covered with lichens and mosses and with "strings" of vegetation growing in crevices in the bedrock. Part of the area had a patchy vegetation of Potentilla fruticosa and Juniperus-bushes (Fig. 1).

The following habitats were studied:

(a) S.-exposed part of a 35 cm wide vegetation string, dominated by Thymus serphyllum L., Helianthemum oelandicum, Festuca ovina and lichens. The lygaeid fauna was rich in species, many occurring in abundance.

(b) N.-exposed part of the same vegetation string. Mostly the same lygaeid species occurred here, but in lower abundance.

(c) On the surface of the bedrock under a 1 cm thick layer of Cladonia pyxidata var. poecillum. A sparse population of Plinthus pusillus occurred here, but the habitat was occasionally visited by many other species. In the following, this habitat is called "under lichens".

(d) In the middle of a 10 cm thick layer of the moss Pleurozium Schreberi (Brid.) Mitt. under a Juniperus-shrub, N.-exposed. This was a principal habitat for Styngnocos sabulosus, which was abundant here.

The following instruments were used:

Temperature was measured with Pt-resistance thermometers and a battery-operated Withoff 6-point recorder. The overall accuracy of this system was ±0.3°C. When measuring surface temperatures of the bedrock, the probe was shaped as a thin tape measuring 20 x 45 x 0.07 mm, which was firmly taped to the rock. For other measurements the probe was 15 mm long and 3 mm in diameter.

Air temperature and relative humidity at 175 cm height, was measured with a shielded thermo-hygrometer (Lambrecht), which was frequently checked against an Assman psychrometer (Lambrecht), and had an accuracy of ±0.5°C and ±3% R.H. Global radiation was recorded by a Kipp solarimeter connected to a mV-recorder (Kipp & Zonen). Relative humidity in the lygaeid habitats was measured with hair-hygrometers (type Pernix, Lambrecht), 45 mm in diameter. Within the range 25–95% R.H., the accuracy was found to be ±3% R.H. Evaporation was measured with 20 ml Piche evaporimeters, with distilled water and with white standard paper as an evaporating surface. The glass tubes were shielded from radiation of the sun by means of Al-foil.

Wind speed was measured with a hot-wire anemometer (Fuess). All measurements in the lygaeid habitats were made with the probes shielded from the radiation of the sun.

The results from two series of observations are presented here in the form of thermo-hygrometers. From the recordings, hourly mean temperature and relative humidity was computed, and thermo-hygrometers were constructed for air at 175 cm height and for the lygaeid habitats (Figs. 2 and 4).

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*Fig. 2.* Thermo-hygrometers for four lygaeid habitats and for air at 175 cm height. Each point indicates hourly mean temperature and relative humidity during June 9–12, 1963. Time at some points indicated in MET.
Fig. 3. Hourly temperature recordings from the four lygaeid habitats and of air at 175 cm height during period June 10, 12 a.m. to June 11, 11 a.m. 1963.

The first period was June 9–12, 1963. Those lygaeid species hibernating as adults occurred as reproducing adults and small larvae of the new generation. The whole period was characterized by a dry, steady, radiation weather. Daily mean global radiation was 426 ly. No rain. Dew fell every night. Mean maximum wind speed at noon was 3.0 m/sec. In Fig. 2, some principal differences between the lygaeid habitats will be seen. The habitat in moss showed small temperature and humidity variations. During the night, this habitat was the coolest. It was isolated from the underlying limestone bedrock by an air-filled moss-layer, 5 cm thick. Thus, it seems that little heat was conducted from the bedrock—which had stored heat during the day—up into the middle part of the moss. Measurements showed that the minimum temperature of the bedrock surface was in fact 3°C higher than in the middle of the moss-habitat. Outgoing radiation resulted in low surface temperatures of the moss during the night. During the day, the moss habitat was shielded from most of the global radiation owing to the Juniperus-shrub. Maximum temperature during the period was 22.5°C. The R.H. was very high even during the warmest part of the day. During the night the moss absorbed water from the dew, and this seemed to evaporate rather slowly during the early day. However, at the moss-surface, the R.H. was found to be about 50% during most of the day.

Both sides of the vegetation string gained heat conducted from the bedrock during the night. This led to higher minimum temperatures, these being about 6°C higher than the air minimum. In the early morning, both sides of the vegetation showed a rapid rise in temperature. The S.-side was shadowed by the vegetation of the N.-side from 09.00 to 11.00,
The prevailing winds, blowing from NW, may explain the lower R.H. at the N-side during part of the day.

It will be noted that the shape of the thermo-hygrograms for both sides of the vegetation is characteristic of a habitat that warms up rapidly, and dries out at a low or moderate rate.

This is in sharp contrast to the exposed conditions under a thin layer of lichen on the bedrock (Fig. 2). During the night, the R.H. was very high. During the early morning, evaporation was very rapid, as a result of the combined actions of wind and a rise in temperature. During this time of the day, the moderation effect of the underlying bedrock is evident. When the lichen layer is dry, a more or less rapid temperature increase will follow (at 7 a.m. in Fig. 2). During the day, much heat is conducted down into the bedrock, and the maximum temperature reached is distinctly lower than those reached in the litter of the S-exposed vegetation.

The shape of this thermo-hygrogram is characteristic of a habitat that dries up rapidly in the morning, chiefly through wind action. In the open habitats of the Alvar, the evaporative action of the winds is high even at very small height above the ground. This can be illustrated by a series of evaporation measurements taken on the bedrock and the two height measurements above the bedrock just discussed. The figures give the total evaporation in ml during June 10, with hourly maximum values in parentheses: At 175 cm: 12.0 (1.65); at 100 cm: 12.0 (1.30); at 1 cm: 8.35 (0.80), and at 0.5 cm: 5.6 (0.65).

The temperature conditions in the different habitats can be studied in more detail in Fig. 3, giving values for a 24 hour period. The moderating effect of the limestone bedrock is evident.

The other observation period was August 10–12, 1964. At this time most lycæids occurred as larger larvae or adults of the new generation. The weather during the whole period was cloudy, daily mean global radiation was 101 ly. The air was humid. No rain. Mean maximum air temperature 17°C, and mean minimum 12°C. No dew. The wind was more intense, with mean maximum at noon of about 5.5 m/sec.

Fig. 4 shows the conditions at the same sites as in Fig. 2 (the probes in the N-side of the vegetation string were damaged by animals). Naturally, the temperatures are generally lower, but it is interesting to note that the general shapes of the thermo-hygrograms are the same as during the more intense radiation weather in June.

However, the drying-up effect of the wind is stronger. This, and the fact that no dew fell during the nights, may explain why the R.H. attained the same low values as in the June observations, despite the fact that the temperatures are much lower.

**DISCUSSION**

28 lycæid species have so far been found on the Great Alvar. 19 species occurred as breeding populations. Of these, 15 species can be considered as characteristic of the Alvar habitats. With the exception of *Pionosomus trichopterus*, all the species found also occur in other habitats on Öland outside the Alvar. In such habitats they are often found in much greater numbers. The only exceptions from this are the Carex-living *Cynus gladiolus* and *Pachybrachis fisticollis*. This applies even to the oligophagous *Macroplax preysleri*, which is very characteristic of the vast areas of *Helianthemum* vegetation on the Alvar, but this species occurs in
much greater abundance on *Helianthemum* on moraine ground in other parts of the island.

It is interesting to note that most of the species found are especially abundant in sandy habitats, especially sand-dunes, sandy heaths, and arable land. Wahlgren (1917), dealing with most insect groups, also found that the ground-living fauna of the Alvar, had a strong resemblance to the fauna of sandy fields, sandy shores and dry slopes.

The reason for this is not understood, but concerning lygaeids, some preliminary results indicate that the food supply may be rather low in many Alvar habitats. Qualitative, as well as quantitative sampling of lygaeids shows that the distribution of most species within the habitats are very heterogeneous. Most species live mostly in highly aggregated populations, especially as larvae. The sites where such aggregations occur are characterized by accumulated, wind-blown seeds. Fallen seeds constitute the principal food for most species of ground-living Lygaeidae. Many such places, optimal for seed-accumulation, are mentioned on pp. 116–118 of this paper. However, preliminary investigations seem to indicate that the seed production of some plant species on the Alvar can vary greatly and show marked differences from year to year.

This is in sharp contrast to the conditions in most of the habitats, where most lygaeids attain their greatest abundance, e.g. in sandy habitats with a wide variety of plants having a high production of seeds. Another characteristic feature of the lygaeid fauna of the Alvar is that most species normally occurring in habitats with thicker layers of dead plant material (litter) are lacking from the Alvar. This also applies to most woodland species. For example, the Alvar fauna is almost completely devoid of *Peritrechus, Drymus, Scolopostethus* and *Eremocoris* spp. These species are all very dependent on stable microclimatic conditions (Coulianos, unpubl.) and especially demand a high relative humidity. Microclimatic measurements in different habitats of the Alvar show that such conditions exist, but only on a too “patchy” and small-scale basis to permit permanent populations of such species to become established. However, a more thorough discussion of these problems must be postponed until more information on lygaeid ecology has been published.

The microclimatic observations show that a considerable variation exists in habitats of ground-living Alvar species. Within the sunny, dry and windy climate of the Great Alvar, microclimatic variation permits the permanent existence of such species as *Stygnocoris sabulosus*, which has been shown to need a stable microclimate.

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REFERENCES


— 1917. Ibid. II. Ibid. Bd. 11. No. 1, 1–130.
Studies of Past and Present Changes in Deciduous Forest Vegetation on Öland

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ABSTRACT

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Former methods of arable field and meadow management in the parish of Vickley, Öland, have been ascertained from old maps and documents. 17th century: Large areas of wooded meadows were used to meet the demand for fodder by cattle. 18th century: Expansion of the area of arable land was efficiently hindered by the local law against tree felling. 19th century: The number of inhabitants in the parish increased rapidly and the number of cattle grew to double that of the 17th century. The law against felling was abolished in 1801. The demand for cattle fodder grew and was met by an increase in the total field area and by a more far-reaching management of wooded meadows. 20th century: Cultivation of crops for fodder started. The demand for low-productive wooded meadows for cattle raising decreased rapidly and the forest started to recolonize the abandoned arable fields and meadows. The forest invasion seems to have been initiated by Betula-Crataegus-Poanus, followed by Quercus-Ulmus-Fraxinus-Corylus. The stabilization of the Betula-Quercetum melicetosum after the cessation of grazing and hay harvesting probably requires at least 70 years; the Ulmus-Fraxinetum probably needs a somewhat shorter time.

Recent changes of vegetation in the deciduous forests were followed between 1955 and 1971 in 70 permanent sample plots. Periodical changes in environmental conditions such as isolated years of drought had a strong influence on forest dynamics. The field layer was more sensitive to changes than the bryophyte bottom layer. Sociological structure was easily disarranged and classification was rendered more difficult due to isolated years of drought or as a consequence of clearing. The management of dense deciduous forests which aims to restore a former open landscape-type should be carried out by light annual thinning over a long period of time. Large scale infrequent clearing will generally induce an invasion of species from other plant communities, causing long lasting instability of phytocoenoses with little resemblance to the former wooded meadows.

INTRODUCTION

An investigation into the ecology and sociology of deciduous forest vegetation on Öland started in the 1950s. The initial work comprised classification of communities and correlation of the presence of single species and groups of species with environmental conditions. The deciduous forests of the island can nearly all be classed as very heterogeneous. Communities on the level of association and alliance rarely cover large continuous areas. In the first stage of the investigation, studies of successional aspects were initiated. A series of permanent sample plots became necessary to follow recent changes in the vegetation of different forest types.

In the 1960s plans to designate deciduous forests on Öland for protection became more definite and urgent. Consequently problems concerning the management of such areas appeared. It was looked upon as desirable to preserve open forests as remains of an extensively used former landscape. However, these remains were found to be both rare and small and in order to restore the open landscape type, management of closed forest was introduced. The methods of carrying out these activities needed study. The consequences of clearing and grazing had to be documented.

Since 1969 the Swedish National Environment Protection Board sponsored investigations into such methods for the management of deciduous forest vegetation. From an early stage the work comprised obtaining facts about the previous structure, area, and management of the forests on the island from old maps and documents. These studies were concentrated on the parish of Vickley. In the present paper results of investigations (permanent sample plots) which were initiated 20 years ago are also communicated. However, field work and discussion of results became intensified after 1968.
Stages in the development of the forests in Vickleby parish back to the 17th century were studied by U. Ekstam. The work on classification of plant communities and on changes of vegetation in permanent sample plots was carried out by E. Sjögren.

DECIDUOUS FORESTS OVER THE LAST FOUR CENTURIES IN THE PARISH OF VICKLEBY

An investigation of the deciduous forests of Vickleby started in 1969 with a sociological study of plant communities within an area of about 220 hectares. The work was centred on problems connected with the development of these forests during the last four centuries, with the object of obtaining information on their areal and structural changes from geometrical maps and parcel maps. The paper by Malmström (1939), “Halländs skogar under de senaste 300 åren”, was a substantial inspiration to this work. Changes in the deciduous forests were correlated with successional changes in the history of culture of arable fields, meadows and pastures.

The parish of Vickleby consists of a western part with fields and forests and an eastern part without forests, dominated by the limestone heath called the “Alvar”. These parts are separated by an escarpment. The heath was the outfield area (utågomark) or common land, which was grazed for the major part of the year. The western part was the infield land (inågomark) with arable fields, meadows (wooded meadows) for hay harvest and short-term annual grazing, and dense unmanaged deciduous forests. In the infield area conditions are favourable for the growth of deciduous tree species with high nutritional demands. Moraine clay rich in lime with few stones predominates (Munthe, 1905). Between the central escarpment and the strait of Kalmarlund two parallel post-glacial gravelly shore-lines known as the Litorina and the Ancylus ridges run in a N-S direction. Close to the shore is an area, about 500 m wide, with gravel from slate bedrock, offering poor conditions for forest growth.

The former distribution of arable fields, meadows and range areas on Öland has been discussed by Göransson (1969, p. 63 ff.). The subdivision of land into arable and common land may have been established as long ago as the early Middle Ages. For example, the limestone heath of Vickleby has probably remained common land for grazing since the 12th century. The arable fields and meadows (wooded meadows) of the villages were gathered in the gärde. In Vickleby this was situated to the W. of the escarpment, where only one small part to the SW. was used as outfield (cf. Fig. 1a).

17th century

The geometrical maps provide information about the distribution and structure of the forest. The first maps of this kind were drawn for taxation purposes. They date back to 1641 and were made by the surveyor Ambjörn Larsson (Kungliga Lantmäteristyrrelsens arkiv, Stockholm [here abbreviated to KLA]; G. 103, Vickleby. G1: 177–182). The following survey for taxation (KLA; G. 103. Vickleby. G3: 477, 481–492) was also found very useful. This map was drawn after wars, in the year 1682 (cf. map “1641”, Fig. 1a). Both maps show that the villages of Karlevi, St. Vickleby, L. Vickleby and St. Frö in Vickleby parish were situated in the same places in the 17th century as they are today. The boundaries between the villages also seem to occupy the same position today judging from topographical features on the maps. Only the boundary between St. Frö and St. Vickleby was not drawn, being established towards the end of the 18th century (KLA; G. 103. Vickleby. 8°, 9°).

The original purpose of these old maps was not to show the distribution of forest. The situations of fields are drawn in a precise way, whilst the areas of forest are drawn with symbols of trees. It is not clear, however, if the surveyor intended illustrating the different densities of the forests by his close and wide spacing of the symbols. The maps seem to be most exact close to villages. Coastlines are not drawn on the map of 1641.

The areas of meadows and closed forest phytocoenoses can be judged from descriptions about the quality of the meadows on the maps. On the “St. Vickleby” 1682 map, was stated: “The meadow is large and reaches the coastline. Parts are, however, covered by dense forests and are impossible to harvest.” From the map of “Karlevi” 1682: “Meadows are covered by dense forests of oak, hazel and lime”. These tree species are the most frequently mentioned ones on the maps. It is probable that dense deciduous

Fig. 1. (a-d) Redrawings from geometrical and survey maps of areas of arable land (hatching) and forests and wooded meadows (tree symbols), in the parish of Vickleby (U. Ekstam).
forest phytocoenoses covered larger areas than managed wooded meadows during the 17th century.

The management of meadows in deciduous forests has been described by Sjöbeck (1933, 1936), Romell (1951) and Selander (1955). These meadows were harvested to provide winter fodder for the cattle. Hay harvest time was late summer. Young branches of trees and shrubs were also cut for fodder. Grazing of the meadows started after the harvest of the arable fields, as fences were rarely present on arable land. Trees in the meadows also provided timber and firewood. Species of trees and shrubs with nutritious leaves and seeds were favoured in the managed meadows and geometrical maps indicate that oak, hazel and lime were most favoured as they were economically important in these respects. The selection of trees and shrubs in former times probably influenced the composition of deciduous forests in south Sweden until recent times.

The productivity of the meadows was estimated on the maps in number of waggon-loads of hay. Variations in the productivity of the meadows from year to year, however, reduce the possibility of obtaining information about the size of meadow areas from such figures. The dry meadow type with oak and hazel (hardeall), is more frequently noted on the geometrical maps than the moist type (sidhalla).

The number of cattle in the Vickleby parish shortly after 1677 was 409 and the number of horses was 65 (cf. Virsell, 1956). There were thus high demands for fodder, which had to be provided for from the hay harvest of the forest meadows. The total area of arable fields in the parish in 1641 was 105 hectares, only about 14 % of the present area. Agriculture was based on animal husbandry and the yield of the meadows in the area was consequently most important.

The supply of timber and firewood from the meadows and forests of Öland was restricted. Forest ordinances in 1569 banned all tree felling without special permission from the authorities. Öland became a private royal hunting ground (cf. Areén, 1924; Landin, 1967). A survey document of 1733 from the village of St. Frö stated: “Oak-forest is scarce in the arable land. Felling of trees is not allowed so both timber and firewood have to be brought from other places.” The law against felling (djurgrädsinrättningen) had a wide conservation effect on the cultivated landscape for 232 years until 1801 when it was abolished.

18th century

Surveys (ravinningar) were made in the parish in 1733 (St. Frö and Karlevi), 1738 (St. Vickleby) and 1754 (L. Vickleby) (cf. KLA; G. 103. Vickleby. 2, 4, 4', 7', 7'', 8'). The distribution of fields and forests has been redrawn in the “1733” map (cf. Fig. 1b). Information on these maps is more detailed than on the 17th century maps. The area of new arable land added since 1641 is small. The expansion of arable land in forests and the expansion of meadow land with scattered trees and shrubs was apparently efficiently stopped by the law (see above).

Some facts have been extracted from descriptions added to the maps. On the St. Vickleby, 1738 map, it is stated about areas E. of the Ancylus ridge: “Meadow in part densely covered by hazel, hawthorn, lime and oak; only half the area can be used for hay harvest” (productivity was estimated to 1/2 palm of hay per hectare). Also, to the W. of the Ancylus ridge it is stated: “Almost the whole area is covered by oak, hazel, lime and hawthorn. Not more than 1/3-1/4 palm of hay can be obtained from one hectare.” Similar facts about the tree layer are provided on the other village maps. There were evidently small changes of area and structure of the forests in the cultivated landscape since the middle of the 17th century. Half the area of the forest was probably only slightly influenced by man and the other half had the character of wooded meadow, managed in various ways.

Valuable information about the 18th century forest was also extracted from the parcel maps of 1773 (St. Frö), 1775 (St. Vickleby) and 1779 (Karlevi) (cf. KLA; G. 103. Vickleby: 2, 4, 8). Forests are drawn with the same symbols as on earlier maps. A classification of the meadows was carried out at that time in order to determine the distribution of the arable land among the farms and to assist with the consolidation of holdings. Documents attached to these maps describe most of the meadows of St. Vickleby as dry meadows, accounting for 66% of the total meadow area. Meadows with “stagg” (probably Nardus stricta) accounted for 22% and moist meadows accounted for 12%.

The dry wooded meadows were restricted to a landscape with oak-hazel communities (Sjöbeck, 1933, 1942). The Betulo-Quercetum melicitosum Sjögren 1971, described below, probably originated from these dry wooded meadows. The moist meadow type was probably the precursor of the Carex elongatae- Alnetum (glutinosae) W. Koch 1926. The
Nardus stricta meadow type (cf. Fig. 3) is today restricted to small areas with a vegetation closely related to the Deschampsia caespitosa-Festuca rubra association (cf. Kielland-Lund, 1971). Nardus dominated meadows with a few other species of herbs. These meadows may have gradually developed in areas where the hay harvest was not very profitable and where annual harvesting produced an impoverishing effect.

Linnaeus visited Öland in 1741. However, in his descriptions from Vickleby (4th June), Nardus was not mentioned. He writes: “The route ran through the most beautiful groves we ever saw which in loveliness by far surpassed all places in Sweden and rivalled all in Europe; they were composed of lime, hazel and oak with an even and green sward without stones or mires; here and there we saw the finest meadows and fields.” Species mentioned by Linnaeus were: Milium effusum, Dentaria bulbifera, Mercurialis perennis, Allaria petiolata and Primula farinosa. All these species except Primula are more or less restricted to the ass. Ulmo-Pratetum, only occurring in the central parts of the island (Sjögren, 1964, p. 19). Primula farinosa belongs sociologically to moist meadows frequently dominated by Sesleria caerulea (Sterner, 1938, p. 138).

The area of arable land increased only slightly after the time when farm consolidation took place. The total area, however, remained comparatively small (KLA; G. 103. Vickleby: 4°, 8°, 8').

19th century

Material for the “1842” map (Fig. 1.c) was to a large extent extracted from the map “Generalstabens topografiska karta över Sverige” sheet 17 Kalmar (1841 and 1842), revised 1866. This map was drawn to the scale 1:100,000, and in order to obtain more detailed information it was necessary to study it in combination with two survey maps of St. Frö and St. Vickleby from 1824 and 1822 (KLA; G. 103. Vickleby: 2°, 8°). Areas with forests and woods are marked on the topographical maps by symbols, but the survey maps give no information about forests. Information on the areas of arable land, meadow land, and on the number of cattle was available towards the middle of the 19th century from the official state statistics (cf. references).

After the law against tree felling was revoked in 1801 the forests on Öland began to become severely devastated. However, this felling was comparatively small in the Vickleby parish, as the large areas of wooded meadows there were still of great importance to the farmers. Between 1805 and 1880 there was an increase in the number of inhabitants in the parish from 575 to 865 (cf. the official State statistics, 1805 and 1880). Thus the demand for timber and firewood increased. The final phase of land consolidation in the 1820s was followed by the enclosure of the arable land (Göran, 1969, p. 77). The use of the land became more differentiated.

Farming became gradually more profitable (Norland, 1949, p. 53) and led to an increase of the total area of arable land between 1820 and 1887 by 100% (1887: 819 hectares). In spite of this increase, the hay harvest in wooded meadows was still essential to meet the demand for winter fodder. The natural vegetation of the meadows was also needed for autumn pasture. Fig. 2 shows figures on the increase of the area of arable land and the decrease of meadow land. The number of cattle and horses in 1865 was 903. Thus the clearing of dense forest in the parish probably became necessary during the first half of the 19th century. Fig. 2 also shows the stabilization of the size of the animal stock during the 1870s. Meadows W. of the Anculus ridge in Vickleby were probably commonly managed by the farmers between 1866 and the 1890s. Grazing pressure was regulated every year after the hay harvest by the meeting of members of the village to deal with common matters (information obtained from the Vickleby hustämmoröckol (1866–1936).

The agricultural revolution greatly influenced the whole structure of the vegetational landscape. Agricultural improvements became especially effective during the second half of the 19th century when cultivation of crops for grazing started. This cultivation increased and finally accounted for a maximum area of 258 hectares in 1919. As a consequence the harvest of natural vegetation from meadows rapidly decreased from 711 hectares to only 52 hectares (cf. Fig. 2) between 1887 and 1911 and was eventually totally suspended.

The last remaining wooded meadows were finally transformed to unmanaged grazing land (beteshagare).

20th century

The area of arable land and forests are shown on the “1969” map (Fig. 1.d). Grazing pressure in open forests was fairly high between 1910 and 1930 and consequently strongly influenced the further develop-
opment of the deciduous forest phytocoenoses. This grazing influence led to the production of a dense mosaic of open and closed forests, clearly seen on the aerial photograph of 1938 (Rikets allmänna kartverk, Hö 1938: 01: 02) and it has also been described by Sterner (1926, p. 159 ff.). After 1920 grazing pressure in the forests decreased gradually as manured pastures became more important (information given by John N. Johansson, Vickleby). In 1937 grazing suddenly decreased by 70% in the western deciduous forests, which belong to the village of St. Vickleby. In this year a large area of the village was purchased by the Swedish Sugar Company Ltd. and after this cattle only grazed on cultivated pastures.

The decrease of grazing in the forests allowed a rapid and dense growth of tree and shrub shoots to develop. A closing of the open cultivated landscape also took place in abandoned arable fields. In addition the landscape of the parish changed when cultivation of conifers started at the beginning of this century. Cultivation of pine had already begun in the 1880s on the land of the Karlevi village (Danielsson, 1918).

Classification of the plant communities of the cultivated landscape of today provides information on present conditions. It can be attempted, however, to ascertain the successional position of a community using information from the history of cultivation. There were several important changes in the use of the forests during the last 150 years, as described above. Consequently the communities described (cf. below) must be looked upon as stages in a long and complicated development. Differential species of deciduous forest communities were described by Sjögren (1964, p. 16 ff.) and revised by the same author in a classification of Scandinavian forest vegetation (Kieland-Lund, 1971). The following associations were distinguished (areal distribution cf. Fig. 3).


4. Deschampsio-Fagetum Passarge 1956. Quercus robur–Betula verrucosa low forest type with high frequency of Juniperus communis in the shrub layer. Differential spe-

Fig. 2. Number of cattle and horses (+ + +) and area in hectares of meadows (-----) and arable land (-----) in the parish of Vickleby between the years 1865–1971.

Fig. 3. The forest of Vickleby Strandskog, parish of Vickleby, to the W. of the Ancylus ridge. Plant communities and management of the cultural landscape of today.
supposed pre-stages. Symbols for plant communities were first plotted on an aerial photograph of the year 1963 (63 GH; 085: 19), scale 1: 10 000.

Areas covered by the four types of association in a mature stage of development generally consist of old forest with a sparsely developed shrub layer. One parcel of land in the forest (8°V) belonged to the vicarage of Vickleby and can be traced on old maps to times before 1738. The land was in former times used as wooded meadow for hay harvest and provided 1/2 palm of hay per hectare. It is today covered by well developed Ulmo-Fraxinetum. The number of differential species is large and Mercurialis perennis is the most important dominant species.

Two other parcels of land (7°IV, 7°III) seen from northern part of the map still have remains of the former dry meadow vegetation but also carry well developed Betulo-Queretum melicetosum. The largest area of this association in the parish, however, is now to be found in Lilla Vickleby äng (cf. Fig. 2d), where there are 16 hectares of oak-forest. The tree layer has a mean age of 100 years. The Alnus-dominated moist forest type is well developed close to the coast of Kalmarsund. The Carici elongatae-Alnetum association is, however, now especially well developed in the five northern parcels of land in St. Vickleby (cf. Selander, 1955, p. 306).

It should be stressed that several meadow vegetation species such as Primula veris, Serratula tinctoria, Orchis mascula, Fragaria vesca are still present in nearly all parts of the forest. Some vascular plants from the meadow stage seem to be able to survive without flowering in the dense forest for at least 20 years after the cessation of the management of the original wooded meadow (cf. Tham, 1971). Information on the previous history of a forest area can be obtained by investigations of the frequency of such taxa; an eventual development towards a stabilized dense forest type can be judged from the frequency of the differential species of the associations described above. Betula verrucosa and shrubs such as Crataegus spp., Rosa spp., Prunus spinosa seem to be especially frequent in the first stage of forest invasion of abandoned arable fields and meadows. In several areas this stage has apparently been followed by a more stabilized stage with Quercus-Ulmus-Fraxinus and Corylus avellana predominating.

RECENT CHANGES OF VEGETATION IN PERMANENT SAMPLE PLOTS

A total of 70 permanent sample plots of 1 m² have been followed nearly every year since 1955 (1956). They are situated in various types of deciduous forest vegetation. The purpose was to follow changes in the plant cover, in localities which were thought to exhibit both high and low vegetational stability. Transitional localities between the communities previously classified were often chosen. For example some permanent sample plots were placed in dry localities where scattered hygrophilous vascular plants or mosses were present. In these habitats a year with unusually low precipitation would influence the hygrophilous species more strongly than in a permanently moist habitat. It was considered possible to trace the ecological range of certain species from their survival ability in habitats far from their ecological preference. Critical conditions affecting the survival or not of hygrophilous species were created during the drought years of 1955, 1959 and 1969. In some localities equally unfavourable conditions were created by the felling of trees and shrubs.

Degrees of cover of the species were registered according to the Hult-Sernander-Du Rietz scale (cf. explanation to Table 1). A modification was added: 5 = 50−75%, and 6 = > 75%. The presence of a species with only one specimen was indicated by a 'x'. The degree of cover had to be calculated rather approximately as the number of sample plots was high.

However, by checking the degree of cover in a brief period, it was possible to record and summarise changes in the presence and absence of individual taxa throughout the whole series of sample plots, every year. Correlations of these changes with periodical or gradual changes of environmental conditions

Plate 1

1. Moist deciduous forest vegetation (Carici elongatae-Alnetum) with the dominance of Carex riparia and Filipendula ulmaria. The wide-crowned Quercus robus specimen suggests the earlier presence in the locality of an open stage in the development of the forest. Vickleby Straskskog, Vickleby. July 1967. Photo E. Sjögren.

were made. The disappearance and invasion of taxa have been considered as much as changes of degrees of cover.

The tree and shrub layers have been checked and degrees of cover refer to an area of 10 × 10 m with the sample plot in the middle. To the shrub layer were referred specimens present in a layer between 0.5 and 3 m above the ground. Frequently during the investigation period shrub layer specimens gradually grew into the tree layer. The sample plots were checked in different months in order to record seasonal changes in the plant cover. Registrations of the presence of spring taxa can generally be left until July but not until August. Additional autumn taxa are very rare in the deciduous forests of Öland.

Field layer

In the period of 1955–1971 there were three years of extreme summer drought in southern Sweden: 1955, 1959 and 1969. The influence of these years on the plant cover in the sample plots was obvious. Species of the Querceto-Betuletum melicotosum were favoured and those of the Ulmo-Fraxinetum, which is a less drought-tolerant assoc., were frequently killed or restrained. Species of the Carici elongatae-Alnetum were also restrained but less than those of the previous ass. Several young shoots of Ulmus, Acer, Fraxinus and Viburnum died in the Ulmo-Fraxinetum shortly after the drought years.

Herbs with differential value of the Ulmo-Fraxinetum which showed a rather low drought tolerance were:

- Geum rivale
- Viola mirabilis
- Mercurialis perennis
- Pulmonaria officinalis

Aegopodium podagraria
- Dentaria bulbifera
- Listera ovata

The presence of the following species with differential value of the Betulo-Quercetum melicotosum was favoured shortly after the drought years especially in localities at random in the ecological range of the Ulmo-Fraxinetum:

- Melica nutans
- Veronica chamaedrys
- Convallaria majalis

Melampyrum pratense
- Fragaria vesca
- Maianthemum bifolium

During the period of investigation there were three years, which brought unusually high amounts of precipitation: 1960, 1961 and 1970. After these years there was an increase of degrees of cover especially of the following species:

Juncus effusus
Filipendula ulmaria
Fraxinus excelsior
Mercurialis perennis
Paris quadrifolia

Dentaria bulbifera
- Viola mirabilis
- Aegopodium podagraria
- Pulmonaria officinalis
- Geum rivale

The gradual closing of forest vegetation in localities, where several permanent sample plots are situated, did not bring about very pronounced qualitative or quantitative changes of the species stocks. In places where there is a gradual increase in the degree of cover of Corylus there is usually a clear increase of Ulmo-Fraxinetum species. This development from 1955–1971 was evidently restrained by the periodical changes of climate due to years of drought.

The number of accidentally appearing and rapidly disappearing species in the sample plots was high compared to the size of the permanent species stock. The most rapidly disappearing of the accidental species are those which are least adapted to the habitat conditions provided. A short time colonization can frequently be correlated with periodical changes of environmental conditions. There is, however, also always a colonization and restraint of several species causing a permanent background dynamics which cannot be explained by correlation with environmental conditions.

When changes in the degree of cover of species within a sample plot of only 1 m² are being considered the sizes of the individual species should be taken into account. A change in the degree of cover from 2 to 3 for example of Mercurialis perennis has a less significance than an equal change for Melampyrum pratense, if the change is to be correlated with a variation of environmental conditions. These conditions should naturally also be considered in the moss layer. Dynamic conditions are often efficiently restrained by predominating species hindering the growing of diaspores arriving at a locality. Such species are for example Poa nemoralis and Veronica chamaedrys in the Betulo-Quercetum melicotosum; Filipendula ulmaria, Geum rivale and Lysimachia vulgaris in the Carici elongatae-Alnetum; Mercurialis perennis, Aegopodium podagraria and Paris quadrifolia in the Ulmo-Fraxinetum.

Bottom layer

In the bottom layer Eurychneum, Mnium and Brachythecium species especially were clearly affected by the drought years. The quantitatively suppression of epigeic species of these genera lasted longer after
each year of abnormal drought than could be recorded among vascular species in the field layer of some sample plots. The influence of the drought on the moss cover was different in the total material of plots. The *Eurhynchium* all. (Waldheim, 1944), which is preferentially present in the *Ulmo-Fraxinetum*, survived the drought years in such woods fairly well. In contrast, it was clearly damaged in sample plots located in a mixture of *Ulmo-Fraxinetum* and the more drought-tolerant *Betula-Quercetum melicetosum*. *Mniium, Brachythecium*, and *Eurhynchium* species were damaged by the drought only to a small extent in sample plots in the *Carici elongatae-Alnetum* where the subsoil water level is comparatively close to the ground surface in the forest.

The method of detecting ecological preferences and ranges of bryophytes involves concentrating on dynamic conditions in the moss cover in habitats which even under normal environmental conditions are obviously far from optimal for the development of certain species. Critical conditions determining whether or not these species survive were created by the years of very low precipitation.

In the surroundings of some permanent sample plots a change of environmental conditions was brought about by the felling of trees and clearing of shrubs. It meant an introduction of more extreme local climatic conditions and the effect was very complex. There was a gradual negative influence on the typical south Swedish deciduous forest mosses but the reaction was not as rapid as after one year of drought. In newly cleared areas in forests there is a rapid increase in the degree of cover of especially nitrophilous species in the field layer. Thus at first a shading effect is introduced and remains for 2–3 years. After that period, damage to the *Mniium, Brachythecium*, and *Eurhynchium* species becomes evident; there is a gradual invasion of rather drought-tolerant species such as *Rhytidiadelphus triquetrus, R. squarrosum, Cirriphyllum piliferum* and *Pseudoscleropodium purum*. The years of unusually high precipitation in the area, in 1960 and 1961, caused the suppression of the *Rhytidiadelphus* species and favoured a development of less drought-tolerant species of the genera *Eurhynchium* and *Brachythecium*.

It is not, of course, possible to correlate all changes in the moss cover with changes in environmental conditions. There is always a continuous accidental inflow of diasporous. But this colonization is also important in the detection of the ecological preferences of mosses. Species with weak preference for ecological conditions in a sample plot generally survive only one or a few years. For example, an accidental colonization by such meadow species as *Tortula ruralis* and *Wetzia microstoma* in dense deciduous forests lasted for a much shorter time than that of the ecologically more adapted *Brachythecium rutilulum* and *Eurhynchium striatum*. Their survival in the sample plots was generally only threatened by earlier quantitatively well-established species with equal ecological requirements and tolerances.

The records from the permanent sample plots revealed that a change in the environmental conditions, such as one year of extreme climatic conditions, had a more rapid and obvious effect on plants in the field layer than on the epigeic mosses. The effect was frequently recorded two of three years later in the bottom layer. This effect was also more long-lasting than in the field layer. An imbalance between changes in field and bottom layer was also recorded in sample plots in localities affected by the felling of trees, and in plots where abandoned small meadows have been rapidly colonized by shrubs. Thus strongly drought-tolerant meadow bryophytes were recorded on all kinds of substrata and were looked upon as relicts in deciduous forests, which were kept open as wooded meadows until about 10–15 years ago.

Comments on the sample plots in an *Ulmo-Fraxinetum* wood

The sample plots in Table 1 represent a part of the series of plots. Dynamics as a consequence of the drought years and felling of trees in the wood are obvious. The following species are eliminated or show lower cover degrees after the drought years: *Geum rivale, Paris quadrifolia, Anemone nemorosa, Viola mirabilis, Ranunculus auricomus, Rubus saxatilis* and the mosses *Eurhynchium praelongum* and *Mniium undulatum*. Young shoots of *Fraxinus, Acer* and *Ulmus* became less frequent as a consequence of the drought years. Grass taxa are few in the community; they are more characteristic of the *Betula-Quercetum melicetosum*. The dynamics of the community between the spring and summer are very strong. During spring *Ranunculus ficaria* and *Anemone* spp. frequently predominate. During the summer they are substituted for example by *Mercurialis perennis* and *Geum rivale*.

A rather large-scale clearing in the wood between 1960 and 1962 strongly increased dynamics in the
localities of the sample plots. There was a specially obvious increase of *Geum rivale*, which was clearly favoured by the suddenly increased supply of nutrition. An increase in the degree of cover of this species was noticeable one or two years after the clearing. High cover degrees were maintained until 1971 and were only slightly influenced by the drought years of 1969.

Changes among the mosses in the bottom layer due to the clearings were not very marked. The increase of *Eurhynchium praetorium* was weak and short-lived. In some sample plots there was an immediate decrease in the degree of cover which seems to be a less frequent consequence, if the results from all the sample plots are taken into consideration.

CONCLUDING COMMENTS

The use of bryophytes as environmental indicators has gradually increased during the last twenty years. Taxonomic and ecological knowledge of bryophytes has become increasingly valuable in the modern use of lichens and mosses as indicators of the degree of air pollution round factories and towns. The practical use of bryophytes to determine the presence and relative amounts of heavy metal ions in the air has recently also given good results. In these studies, it becomes increasingly more necessary to know the ecological ranges and preferences of bryophyte taxa. The absence of bryophytes which generally belong to forest phytocenoses, from meadow vegetation round densely populated areas in south Scandinavia, and their frequent presence in meadows close to towns in Ireland and in Britain, should not, for example, be correlated with different degrees of air pollution. Such correlations need a good knowledge of the ecology of the taxa involved. Tolerance and preference for ecological factors such as drought, extremes of temperatures, pH, and substrata, must first be investigated in areas where environmental conditions are only slightly influenced by man. Such areas will be found in areas where nature conservation has helped to stabilize the dynamic changes in phytocenoses over a fairly long uninterrupted period of years.

A knowledge of the ecology of forest mosses is also valuable in other ways in the modern landscape, strongly influenced by man. The comparatively slow dynamic change of a moss carpet on various substrata is useful for the tracing of earlier changes in environmental conditions as a consequence of changes in management. The balance in deciduous forest phytocenoses is easily upset and apparently requires a fairly long period of years to be re-established. Information about successional rate is important in any attempt at preservation and management of these phytocenoses.

The checking of the series of sample plots has revealed that even short-term changes in environmental conditions such as single years of extreme climatic conditions produce a strong increase of dynamics. Phytosociological investigations of deciduous forest vegetation should not be carried out in the two years following a year of such abnormal conditions. The classification of communities is very difficult and the result might be misleading because of the increase in the number of accidentally colonizing species. The stabilization of the moss cover seems to require more than two years.

Single large-scale clearance of trees and shrubs is the method often used in attempting to re-establish a former mosaic landscape of wooded meadows and open forests. The consequences are an enormous increase in dynamics with a more or less accidental invasion of diaspores from several surrounding vegetation types including nitrophilous communities of cultivated land. After about five years a cover of *Corylus* and *Fraxinus* shoots has often developed in an Ulmo-Fraxinetum. A dense impenetrable shrub layer of *Crataegus* and *Prunus* develops in the Betulo-Quercetum melicetosum.

In order to re-attain earlier stages in the development of the forests, annual thinning on a small scale should be carried out by hand, and spread over a long period of years in order to keep the dynamics to a minimum.

The conservation of deciduous forests of varying compositions might be considered as very important. These forests have become increasingly rare all over Europe during the last centuries of industrialization and because of the increase in the area of cultivated fields. They are of extreme value to future investigations in the field of ecology.

ACKNOWLEDGEMENTS

The authors are greatly indebted to the Swedish National Environment Protection Board for financial support. Grateful acknowledgement is made to Professor Per Hallenberg, Zoological Institute of the University of Uppsala, for valuable discussion and great hospitality at the Ecological Station on Öland. We owe a special debt
Changes in deciduous forest vegetation on Öland

REFERENCES


Table 1. Changes of vegetation in permanent sample plots in an Ulmo-Fraxinetum wood

Explanations:
T - tree layer, S - shrub layer, F - field layer, B - bottom layer.
Size of sample plots: 1 m².

Cover degrees: × - single specimen, 1 = ≤ 1/16; 2 = 1/16 -1/8; 3 = 1/8 -1/4; 4 = 1/4 -1/2; 5 = 1/2 -3/4; 6 = ≥ 3/4.
Locality: Village of Stora Dalby, parish of Kastlösa.
Habitats: Granular soil surface (earth-worms). Low light supply to field and bottom layer. Leaf litter from all trees and shrubs except oak and hazel decay rapidly. Litter of ash, elm and maple is nearly always in a minority in the litter layer during the summer and autumn period.
Community: Ulmo-Fraxinetum.

Sample plots: (1) Dominance of oak and hazel leaves in the litter. Clearings in 1961-62. Rapid increase in the field layer cover. (2) Dominance of oak leaves in the litter. Clearings in 1961. Gradual increase in the cover degree of the field layer. (3) Litter from oak, hazel and maple. Clearings in 1960-61. Very rapid increase in the cover degree of the field layer. (4) Litter from elm, ash and oak. Clearings in early 1961. Low cover degrees of tree and shrub layer may have hindered the usual increase in the field layer cover after clearings. Slowly decreasing cover of bottom layer. (5) Dominance of maple leaves in the litter. Clearings in 1961. After 1961 a gradual increase in the cover degree of the field layer. (6) Dominance of oak leaves in the litter. Clearings in 1961. (7, 8) Litter from oak, hazel and elm. Clearings in 1961, followed by a rapid increase in the cover degree of the field layer (Geum rivale) and a slow decrease in the cover degree of the bottom layer (Eurynychium praerectum). (9) Litter from oak and hazel, half from leaves, half from branches. Clearings in 1961-62. Several bryophytes taxa on a silicious stone and on branches. (10) Litter dominated by oak and hazel leaves; elm leaves are few. Clearings in 1961. Rapidly increasing cover of the field layer; slowly increasing cover of the bottom layer severely damaged by drought in 1969. (11) Litter dominated by oak and hazel leaves. Clearings in 1961. Most rapidly increasing cover of the field layer; slowly decreasing cover degree of the bottom layer.

Sheep Grazing and Changes of Vegetation on the Limestone Heath of Öland

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ABSTRACT

Rosén, E. and Sjögren, E. (Institute of Ecological Botany, University of Uppsala, Uppsala, Sweden.) Sheep grazing and changes of vegetation on the limestone heath of Öland. ZOON, Suppl. 1: 137-151, 1973

The sheep preferentially graze the Avenetum and the Festuconem communities on the limestone heath ("Alvar") of Öland. Ungrazed Avenetum: Crop 2 950 kg per hectare (dry weight 1969); crude protein 8.9% and digestibility 61% (1972). Ungrazed Festuconem (same years): 1 330 kg per hectare; crude protein 5.4% and digestibility 50%. Sheep-grazed Avenetum has somewhat higher figures for crude protein and digestibility probably as a consequence of an increase of herbs with creeping growth forms in the plant cover. This may be an adaptation to the permanent wearing of the vegetation caused by grazing.

Avenetum has two annual maxima of productivity, late June to early July and late August to early September. Ungrazed areas of the Avenetum become invaded by juniper. The standing crop of juniper was 3 364 kg per hectare (dry weight, 20°C) in an area where the shrubs had an average age of 63 years. 33% of this weight was accounted for by assimilating parts. Mean snow cover is generally 10-15 cm less in shrubless areas than in a juniper scrub.

Frost-induced soil movements and polygon structures at the soil surface are frequent in the Festuconem. Heavy grazing in the Avenetum has allowed the frost-induced soil movements to increase and has thus rendered the community more susceptible to erosion by the trampling of grazing animals.

Changes of vegetation in permanent sample plots have shown that unusually dry years affect grass-rich communities more severely than others. Grazing pressure in areas rich in the Festuconem should be reduced for two or three years after a year of drought. The average Alvar vegetation can withstand a grazing pressure of 0.63 ewes (each ewe with 1.0 lamb) per hectare. A grazing pressure of 0.4 ewes/hectare should be used to maintain an unaltered stock of animals over a period longer than 10 years. A concentration on high lamb quality is recommended to maintain the sheep grazing on the Alvar profitable. This grazing pressure may also be sufficient to maintain the former Alvar landscape free from dense invasions of shrubs on gravelly ridges.

INTRODUCTION

These investigations were carried out on the calcareous heath "Stora Alvaret" on the island of Öland in the Baltic. The highest altitude of this island is 57 m. The bedrock consists entirely of Cambrian and Ordovician limestone. The surface of the Alvar is slightly inclined towards the E. There is an escarpment, klint (cf. Martinsson 1958, p. 15), along the western borderline of the heath. The Quaternary deposits are unevenly distributed on the Alvar, leaving the limestone bedrock exposed over large areas. The topography of the heath is very level, except in places with low gravelly ridges. Fissures in the bedrock are common and are significant in the drainage of the Alvar. The area of the heath is about 300 km².

Öland has a semi-arid climate. Annual precipitation is only about 400 mm as the island is situated in the rain-shadow of the mainland. Mean temperatures are higher than on the nearby mainland. February is the coldest month with mean temperatures between -2 and -1°C. July is the warmest month with mean temperatures between +16 and +17°C. The winds from the SW. predominate but easterly and north-easterly winds are frequent and strong during the autumn and winter seasons. The number of sunshine hours is 2 000 per year. Mist occurs over the southern half of the island on 15-20% of the days. For further more detailed information on climate and geology, see Sjögren (1961, p. 9 ff.) and Königsson (1968, p. 12 ff.).

The Alvar vegetation has been influenced by grazing probably for thousands of years. Postglacial changes of climate also induced fluctuations in the composition of the plant cover. The vegetation from ancient times until now was probably characterized by a dense mosaic of plant communities, tolerant of dry climatic conditions and also adapted
to the very special environmental conditions offered by the mainly shallow, easily eroded soils on the calcareous plain.

_Avenetum alvarense_ (Albertson 1950, p. 304 ff.) is the predominant association on moraine ridges and in marginal parts of the heath. _Festucetum communis_ (op. cit., p. 289 ff.) predominate on shallow soil and _Sedetum tortellosum_ (op. cit., p. 284 ff.) on bare rock. The _Molinetum alvarense_ (op. cit., p. 310 ff.) occupies moist habitats. The two former associations are referred to as _Avenetum_ and _Festucetum_, respectively in the following account. Trees are rare on the heath but some communities are rich in shrubs such as _Juniperus communis, Prunus spinosa_ and _Potentilla fruticosa_. _Carylus avellana_ occurs only in areas where there are many deep fissures.

The plant communities are rich in taxa with southeastern distributions in Scandinavia. Some foreign taxa occur as outposts more or less isolated from their main distribution such as the West-siberian _Artemisia ruprechtii_, the South-east European-South Siberian _Plantago tenuliflora_, the Mediterranean _Globularia vulgaris_ and _Ranunculus illyricus_ and the Arctic-montane _Poa alpina_. There are also some endemic taxa such as _Helianthemum oelandicum, Festuca rubra var. oelandica_ and _Viscaria alpina var. oelandica_. The Alvar vegetation possesses several endemic plant communities and it may be considered as unique in the world.

Large areas of the Alvar were exploited for sheep grazing in the latter part of the 1950s, and the grazing pressure was very heavy (1 ewe per hectare or more). In 1965 there were around 10,000 sheep grazing on the Alvar. In the autumn of 1967 the Swedish National Environment Protection Board introduced a short-term protection of some of the unexploited parts of the heath, and in 1969 financial support was provided for investigations which could give detailed information on the grazing influence on the vegetation and soil, and which proposed schemes for the management of the protected areas.

Some investigation results obtained from this conservation project (supervised by E. Sjögren) are presented in this paper. Annual regional surveys of the consequences of grazing on the vegetation and soil, including records of permanent sample plots, were carried out by E. Sjögren. Investigations on the standing crop of the preferentially grazed plant communities have been carried out by E. Rosén.

**CONSEQUENCES OF GRAZING ON THE ALVAR HEATH**

The history of grazing on the large limestone heath of Öland probably goes back thousands of years. Grazing was carried on to a small extent. Shepherds tended the flocks of sheep as there were no fences. Up to the beginning of the 20th century, grazing by young cattle was also fairly common on the Alvar. In recent decades the number of horses has decreased considerably. Cattle stocks were also moved away from the meagre pastures on the Alvar outfield areas. Up to the 1940s the grazing as a whole was responsible for the absence of dense groups of shrubs and trees on gravelly ridges. In the 1950s heavy sheep grazing began, especially for meat production. Grazing is possible for about 7 months during the summer season. During the rest of the year the sheep flocks are fed in small pens. Water supply has been a major problem in dry years.

The very severe and evident changes of vegetation on the Alvar as a consequence of the heavy sheep grazing called urgently for the adjustment of the grazing pressure to the very special type of vegetation and to the environmental conditions of the heath. Investigations on a small scale were started in 1965. Between 1965 and 1969 changes of vegetation and soil were recorded. In 1969 investigations into the productivity of two communities of the heath (_Avenetum_ and _Festucetum_) were added to this general survey.

Of these communities, the sheep preferentially graze the _Avenetum_. When the shortage of food is more acute they graze _Juniperus communis, Prunus spinosa_ and _Potentilla fruticosa_. The shrub layer is affected by grazing after about three years if a grazing pressure of 1.0 ewe + 1.3 lamb per hectare is maintained. _Juniperus_ regenerates very slowly and _P. fruticosa_ somewhat faster after grazing has ceased in an area. The impact of grazing pressure on the Alvar vegetation is influenced by the fact that between 20–50% of the plant communities in various parts of the heath are of little grazing value to the sheep.

The Alvar vegetation is damaged by the intensive sheep grazing, directly through grazing and trampling and indirectly in several other ways. Not all species tolerate grazing year after year. After a few seasons _Globularia vulgaris, Gypsophila fastigiata, Anthericum ramosum_ and orchids almost disappear. Species with a weak root system are often pulled up by the sheep. Sheep grazing has brought about considerable
manuring over large areas. Several species which are normally seldom found in the Alvar heath vegetation appeared frequently. The natural calciphilous vegetation succumbs to these competitors (see Table 1). Close to pines, there is often a 100 percent-dominance of such nitrophilous species as Cirsium arvense, Polygonum aviculare, Poa annua, Stellaria media, Urtica dioica and Geranium robertianum (cf. Ljung, 1970).

Soil erosion is a most serious harmful consequence of sheep grazing. It is most destructive as the recovery time is very long. The soil cover around Festuca tufts is worn away by the trampling animals. As the bottom layer is an important stabilizing component in all Alvar vegetation, the elimination of mosses and lichens opens the way to the erosion of the vascular plant cover. The cover of mosses and lichens on bare limestone rock is quickly broken up by the grazing flocks of sheep. When the weather is windy and dry, fragmentation of mosses and lichens causes the distribution of a great number of diaspores by the wind.

In the bottom layer as well as in the field layer there are changes in the calciphilous species because of overgrazing. Such mosses as Pohlia nutans and Bryum argenteum, with a wide ecological range, increase explosively to dominance that may be total in some places. In some areas these species give a typical landscape colour to areas that are intensively grazed. As a result of soil erosion species with low competitive ability, such as Erophila verna, Horningia petraea and Bromus hordeaceus, may invade large areas of limestone heath. Plantago lanceolata often colonizes such bare habitats, forming dense carpets.

Intensive grazing on the limestone heath of Öland has now been going on for more than a decade. In areas where the pressure of grazing has been maintained at 1 ewe per hectare including the lambs, the natural vegetation has changed and been destroyed to the extent that it has become very difficult to maintain sheep farming at a profitable level.

preferentially grazed by the sheep, the Avenetum and the Festucetum, were investigated.

Sample plots statistically chosen were located in different parts of the heath. Areas ungrazed by sheep were to be studied for comparison with grazed areas.

Two sample plots of 1 m², a control square and a test square, were located adjacent to each other and were fenced in. The corners of the plots were marked with aluminium posts. A space of 0.5 m was left between the plots and the fence in order to eliminate the influence of grazing sheep. In areas grazed by sheep only half the number of sample plots was fenced whilst the other half was left open for further grazing.

In July an inventory of the vascular plants, mosses and lichens of all sample plots was made. The scale for degrees of cover suggested by Hult-Sernander-Du Rietz (Du Rietz, 1921, p. 225) was used. The test plots were harvested in August rather than in July in order to protect the soil from the usually severe July drought. The plots were harvested with a pair of scissors. Grasses and other herbs were cut about 2 mm above the soil surface. The crop was dried for 24 hours at a temperature of 80°C after sand and fragments of mosses and lichens had been removed. The harvest was repeated in November. The annual harvests were carried out between 1969 and 1972. When the time required for the harvests was known in detail a modification of the harvest was introduced. It was desirable to obtain a series of plots which showed the gradually increasing effects of the harvests, and also a series of plots which allowed the recovery of the plant cover after the cessation of harvest to be studied. The harvests were thus carried out according to a system explained in Fig. 2.

The figures for the two crops from each plot each harvest year (1969–71) were combined.

The mean weights (in g/m²) of dry matter were calculated for the communities Avenetum and Festucetum and are shown below. Abbreviations: G = grazed, UG = ungrazed, F = fenced, UF = unfenced.

<table>
<thead>
<tr>
<th>Year</th>
<th>Avenetum</th>
<th>Festucetum</th>
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<tr>
<td></td>
<td>G</td>
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</tr>
<tr>
<td>1969</td>
<td>176</td>
<td>155</td>
</tr>
<tr>
<td>1970</td>
<td>131</td>
<td>103</td>
</tr>
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<td>1971</td>
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<td>84</td>
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</table>

**STANDING CROP**

Investigations into productivity on the Alvar aimed to obtain measurements of standing crops during the grazing season. It should be possible to make recommendations on grazing pressures from these figures. Two endemic Alvar communities which are
Fig. 1. Consequences of grazing in the years 1966, 1967, 1970, 1971, on the southern limestone heath of Öland. Explanations to the five degrees of change of the vegetation and soil caused by grazing:

1. Slight or non-existent consequences of grazing. (a) Fragmentation by trampling animals of fruticulose lichens, such as Cladonia spp., Cetraria spp., Thamnolia vermicularis, cannot be observed or is very rare. (b) No wearing away from the limestone rocks of crustaceous lichens, such as Verrucaria spp., Lecanora spp., or of foliaceous lichens such as Collema spp. (c) Fragmentation of cushion-forming mosses, such as Tortella spp., Schistidium apocarpum, Ditrichum flexicaule is not observed or is rare. (d) High frequency in the Sedetum tortellosum of
brightly coloured crustaceous lichens, such as *Toniinae coeruleo-nigricans*, *Lecanora* spp., *Lecidea decipiens*, *Fulgensia* spp. (German: Bunte Erdflechten). (c) Flowering and fructification of vascular plants not restricted. (f) No or very slight grazing of shrubs (*Prunus spinosa*, *Potentilla fruticosa*, *Juniperus communis*, *Rosa* spp.). (g) Accidental presence of nitrophilous weeds from fields and cultural pastures. (h) Erosion of plant cover by trampling of grazing animals not observed. (i) Manuring by grazing animals is slight. (j) Polygon structures at the ground surface are not disarranged. (k) Grazing is strongly concentrated to *Avenetum* vegetation on gravelly ridges.

2. Small changes by grazing. (a) Fragmentation of lichens is rare. (b) Slight wearing away of crustaceous lichens. (c) Few fragmentated moss cushions. (d) Brightly coloured crustaceous lichens are rather frequent.
Flowering and fructification slightly restrained in the Avenetum community. (f) Local rather heavy grazing of shrubs. (g) Nitrophilous weeds are not frequent. (h) Erosion caused by grazing animals is very slight. (i) Local but rather considerable manuring by grazing animals. (j) Polygon structures slightly disarranged. (k) Grazing mainly takes place in the Avenetum vegetation.

3. Rather far-reaching changes caused by grazing. (a) Fragmentation of lichens occurs frequently. (b) Local wearing away of crustaceous lichens. (c) Several moss cushions fragmented, displaced or turned upside-down, and thus moribund. (d) Brightly coloured lichens are rare. (e) Flowering and fructification restrained in all plant communities. (f) Locally scattered shrubs killed by grazing, over large areas. (g) The Avenetum shows a frequent immixture of nitrophilous species. (h) Bare patches in the plant cover caused by trampling are rare. (i) Heavy manuring in all plant communities. (j) Polygon structures deranged over large areas. (k) Grazing rather evenly distributed between plant communities.

4. Far-reaching changes caused by grazing, locally reparable in the bottom layer only after about 50 years’ cessation of grazing. (a) Fragmentation of lichens in all plant communities over large areas. (b) On all bare limestone rocks, far-reaching wearing away of lichens. (c) More than 75% of moss cushions disappeared or dislodged. (d) Brightly coloured lichens in Sedetum strictum very rare. (e) Restraint of flowering and fructification in all plant communities. (f) Over large areas, shrubs heavily grazed and specimens of Potentilla, Primula, and Juniperus killed. (g) Very frequent immixture (local predominance) of nitrophilous species in the Avenetum. (h) In all plant communities bare mineral soil patches in the plant cover, caused by trampling. (i) In all plant communities considerable manuring. Sheep manure is present in every dm² in the Avenetum. (j) All polygon structures deranged or regionally deranged. (k) Grazing pressure evenly distributed between plant communities.

5. Very far-reaching changes caused by grazing over large areas (bottom layer) reparable after more than 50 years’ cessation of grazing. (a) Over large areas, far-reaching fragmentation of lichens, fragments blown away or trampled down into the mineral soil. (b) Crustaceous lichens on the limestone rock totally worn away. (c) Nearly 100% of moss cushions disappeared. (d) Brightly coloured lichens in moss cushions nearly absent. (e) Flowering and fructification heavily restrained in all plant communities. (f) Killing of shrubs over large areas. (g) Nitrophilous weeds have invaded the Avenetum and provide more than 50% of cover in large areas. A few vascular plant species of the original Avenetum have survived. (h) Bare patches in the plant cover caused by trampling are frequent in all plant communities. Erosion of mineral soil is considerable locally. (i) Considerable manuring in all plant communities. (j) No polygon structures remain. (k) Grazing pressure nearly equally distributed on different plant communities. Weak grazing preference for the Avenetum due to the invasion of fast-growing nitrophilous species.

In 1969 the groups of fenced and unfenced plots each comprised 5 plots in grazed areas with Avenetum and Festucetum; there were 20 plots respectively in ungrazed Avenetum and Festucetum vegetation (60 sample plots in total). In the following years the size and number (Fig. 2) of harvested plots was modified.

The amount of crop obtained in 1969 was considerably larger in ungrazed than in grazed Avenetum. This was expected as the sheep consumed some fodder in 1968 which was not included as dead material in the figures of 1969. In contrast, there were small differences between grazed and ungrazed Festucetum vegetation. In 1970 all the figures are much lower as a consequence of the previous harvest and removal of crop in 1969. The decrease was especially important in the Festucetum, and was of equal size in grazed and ungrazed areas. In the Avenetum the strongest decrease was obtained in ungrazed areas and this decrease was accentuated in 1971. There was not an equally high decrease in the harvested standing crop from grazed Avenetum plots, as the vegetation there due to the grazing seems to have become more tolerant to the wear caused by clipping and to the removal of crops. In ungrazed Festucetum plots there was a further decrease of standing crop in 1971 which could not be found in grazed plots in this community. No large differences were recorded in the number of taxa or in the qualitative changes of the stock of species between grazed and ungrazed plots. However, the Avenetum is generally composed of a larger number of taxa (40) than the Festucetum (20).

Table I. Changes of vegetation due to differential grazing pressure illustrated by sample plots placed at different distances from a sheep pen on the Åker heath of Öland.

Explanations

Size of sample plots 1 m². Four sample plots at each distance from the sheep pen.

Degrees of cover according to Hult-Sermander-Du Rietz (° = single specimen; 5 = > 75%)

Locality: Village of Bärby, parish of Mörbylånga.


Grazing pressure per hectare: 1.0 ewe (1965); 2.0 ewes (1966); 1.5 ewes (1967); 0.5 ewe (1968).

Comments: Close to the pen a few vascular plant species, mainly weeds of arable land. No lichens and mosses. Gradual increase in the number of vascular plants, lichens and mosses with increasing distance from the pen. Nitrophilous weeds gradually disappear. Lichens do not appear closer than 200 m from the pen.

Recordings were made by Elisabeth Ljung, Institute of Ecological Botany, University of Uppsala.
<table>
<thead>
<tr>
<th>Sheep grazing and vegetation changes on Öland's Alvar</th>
<th>143</th>
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</thead>
<tbody>
<tr>
<td>Table 1: Relative percentage of various species to the total number of species in different locations.</td>
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</table>

<table>
<thead>
<tr>
<th>Location</th>
<th>Percentage (%)</th>
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<tbody>
<tr>
<td>Location A</td>
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</tr>
<tr>
<td>Location B</td>
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</tr>
<tr>
<td>Location C</td>
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<td>Location D</td>
<td>1.2</td>
</tr>
<tr>
<td>Location E</td>
<td>0.9</td>
</tr>
</tbody>
</table>

| Table 2: Distance from the sea (km) | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 |
|---------------------------------|---|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| Percentage of various species   | 3.5| 2.1| 1.8| 1.2| 0.9 | 1.5| 2.0| 2.5| 3.0| 3.5| 4.0| 4.5| 5.0| 5.5| 6.0| 6.5| 7.0| 7.5| 8.0| 8.5| 9.0| 9.5|10.0|10.5|

| Table 3: Percentage of various species to the total number of species in different seasons. |

<table>
<thead>
<tr>
<th>Season</th>
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<td>Summer</td>
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<tr>
<td>Autumn</td>
<td>1.8</td>
</tr>
<tr>
<td>Winter</td>
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| Table 4: Percentage of various species to the total number of species in different weather conditions. |

<table>
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<tr>
<td>Cloudy</td>
<td>1.8</td>
</tr>
<tr>
<td>Snowy</td>
<td>1.2</td>
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</table>

| Table 5: Percentage of various species to the total number of species in different soil types. |

<table>
<thead>
<tr>
<th>Soil Type</th>
<th>Percentage (%)</th>
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<tbody>
<tr>
<td>Sandy</td>
<td>3.5</td>
</tr>
<tr>
<td>Clay</td>
<td>2.1</td>
</tr>
<tr>
<td>Loamy</td>
<td>1.8</td>
</tr>
<tr>
<td>Silt</td>
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| Table 6: Percentage of various species to the total number of species in different management practices. |

<table>
<thead>
<tr>
<th>Management Practice</th>
<th>Percentage (%)</th>
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<tr>
<td>Grazing</td>
<td>3.5</td>
</tr>
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<tr>
<td>Welding</td>
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| Table 7: Percentage of various species to the total number of species in different water availability conditions. |

<table>
<thead>
<tr>
<th>Water Availability</th>
<th>Percentage (%)</th>
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<tr>
<td>Dry</td>
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<tr>
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<td>Flooded</td>
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| Table 8: Percentage of various species to the total number of species in different pest and disease pressures. |

<table>
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<tr>
<th>Pest and Disease</th>
<th>Percentage (%)</th>
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<tbody>
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<td>None</td>
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<tr>
<td>Light</td>
<td>2.1</td>
</tr>
<tr>
<td>Moderate</td>
<td>1.8</td>
</tr>
<tr>
<td>Severe</td>
<td>1.2</td>
</tr>
</tbody>
</table>

| Table 9: Percentage of various species to the total number of species in different human activities. |

<table>
<thead>
<tr>
<th>Human Activity</th>
<th>Percentage (%)</th>
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<tr>
<td>Light</td>
<td>2.1</td>
</tr>
<tr>
<td>Moderate</td>
<td>1.8</td>
</tr>
<tr>
<td>Severe</td>
<td>1.2</td>
</tr>
</tbody>
</table>

| Table 10: Percentage of various species to the total number of species in different bird and mammal pressures. |

<table>
<thead>
<tr>
<th>Bird and Mammal</th>
<th>Percentage (%)</th>
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<tbody>
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<td>Light</td>
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<td>1.8</td>
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<tr>
<td>Severe</td>
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</tbody>
</table>
Grasses, other herbs and dwarf shrubs

In 1970 the harvested material from sample plots was separated into grass, dwarf shrubs and other herbs. *Helianthemum* spp. and *Thymus serpyllum* were included in the dwarf shrubs. The percentage of each category in the *Avenetum* and the *Festucetum* (weight of dry matter) after one year of harvest was (abbreviations see Table p. 139):

<table>
<thead>
<tr>
<th></th>
<th><em>Avenetum</em></th>
<th></th>
<th><em>Festucetum</em></th>
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<td>G</td>
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<td>shrubs</td>
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</table>

Herbs (except grasses) predominate clearly in grazed *Avenetum*, and to a somewhat smaller extent in the ungrazed community. The difference in the percentage of herbs (except grasses), between the grazed and the ungrazed *Avenetum* is probably usually more pronounced than shown by the figures, as a consequence of a previous harvest of the plots in 1969. A further investigation in 1972 has shown that the percentage of grasses and other herbs in ungrazed, non-harvested areas is nearly equal. Dwarf shrubs in such areas are present and account for 15–20%.

The results suggest that the herbs (except grasses) tolerate grazing better and also adapt more rapidly to creeping forms. The grasses may be more effectively restrained due to their more erect growth form. Grasses predominate in the *Festucetum* plots, partly as a consequence of special environmental conditions, especially the thin soil cover (cf. below), prevailing in the habitats of the community. The percentage of grasses was higher in unfenced plots in the grazed area, which may indicate that herbs (except grasses) are preferentially grazed. These have the opportunity to recover in the fenced plots. In all plots in both communities there was a pronounced decrease of dwarf shrubs as a consequence of grazing and harvest.

Because of the preferential grazing of the sheep it was decided to enlarge the productivity investigations. Five sample plots were harvested once a month from May to November (1972) in grazed and ungrazed *Avenetum* and also in ungrazed *Festucetum*. Mean values for the season of dry matter, crude protein and digestibility were determined (%):

<table>
<thead>
<tr>
<th></th>
<th>Crude protein</th>
<th>Digestibility</th>
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<tr>
<td><em>Avenetum</em> (ungrazed)</td>
<td>8.9</td>
<td>61</td>
</tr>
<tr>
<td><em>Avenetum</em> (grazed)</td>
<td>9.7</td>
<td>63</td>
</tr>
<tr>
<td><em>Festucetum</em> (ungrazed)</td>
<td>5.4</td>
<td>50</td>
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</tbody>
</table>

No significant differences between different months were obtained. Values both for crude protein and digestibility are higher in the *Avenetum* than in the *Festucetum*. The highest energy content is thus to be found in the *Avenetum*. The reason why the highest values are found in the grazed *Avenetum* may be explained by its possession of a lower per-
centage of grasses and a considerable supply of nitrates. Values are not unexpectedly high or low if compared with those obtained from similar types of dry vegetation in Sweden.

**Seasonal changes**

A special investigation was introduced in 1972 to obtain information on the development of the plant cover through the summer. 10 plots (25 × 25 cm) in a homogeneous *Avenetum* were harvested in May, and each following month 10 new plots were harvested until October. The material was separated into dead and living dwarf shrubs, grasses, and other herbs. Dwarf shrubs were dealt with after a separation into current shoots and older parts.

Standing crops as well as the proportion of living plants have maxima in late June to early July and in late August to early September. There is a decrease of productivity in July, probably as a consequence of low precipitation. There is also a loss of field layer leaf and seed litter in July. The obtained values for dead plants show that in August there is a large supply of species which started to fade in July–August. Living herbs (except grasses) are the most important in the community productivity in June and July, but at the end of August and beginning of September there is a dominance of grasses and dwarf shrubs. In September all productivity values decrease except values for the proportion of dead material which gradually increase. Standard error of the mean values were about 10%. Figures obtained in August and September may be above normal due to high precipitation at the beginning of August.

If these values are compared with the results of the harvests from sample plots carried out in August it may be suggested that there has been a partial loss of standing crop as a consequence of the late harvest time. The harvest material obtained in November, however, shows rather well the real amount of material produced between August and November as only a little dead vegetation is accumulated in the period of time following the August harvest. The whole annual production above the soil is thus not obtained from the two harvests although the most part is. The sample plots are useful when comparing the standing crops of different years, taking into consideration changes of vegetation caused by harvest wearing.

**Juniper scrub**

In areas on the Alvar with a deep soil-cover a low grazing pressure results in a more or less strong invasion of *Juniperus* shrubs. The standing crop of the juniper specimens in a densely overgrown area was studied in 1970. Two adjacent sample plots of 50 × 50 m were used and the vegetation was mapped in detail. The height and size of each juniper were measured and a classification into size groups was made. The junipers in one of the large sample areas were cut down. The total number of shrubs was 130. The fresh weight of the shrubs was measured and 20% was kept and dried in order to obtain dry weight measurements after half a year of drying at about 20°C. According to the figures obtained the fresh weight of the junipers was 5 100 kg and the dry weight 3 364 kg per hectare. 33% of the dried material was accounted for by needles and current shoots; the rest was bark and wood. The mean age of the juniper stand was 63 years.

Under most of the cut-down shrubs was a thick litter of needles, mostly without a plant cover. The colonization of these needle areas has been recorded in 1971 and 1972 to obtain information on rates of colonization. The clearing of shrubs over a large area of the Alvar followed by heavy grazing pressure, would probably promote soil erosion, initiated in the loose needle carpets. It is thus necessary to begin grazing within a cleared area with a gradually increasing animal stock.

In March 1969 and 1970 winter investigations began. The snow cover was found to be very thin.
in those areas grazed by sheep and lacking a shrub layer, compared to ungrazed scrub vegetation. The snow depth was measured in 1971 in the two large sample areas described above. In the uncleared area there were high snow-drifts close to junipers. The mean snow depth was 33 cm. The snow cover of the cleared area was much more evenly distributed and the mean depth was 22 cm. The difference was hence about 1100 m³ of snow per hectare. The snowdrifts with a generally very densely-packed snow gives a longer lasting protection to the vegetation, and this is important in early spring when diurnal changes of temperatures are large close to the ground. The snow disappears generally 1–2 weeks earlier in the shrubless Alvar than in the juniper scrub.

In March 1972 snow cover on the Alvar was very thin. Frost-induced soil movements in the thin soil cover were found to be considerable. Such disturbance is especially severe for the vegetation on thin gravelly soil layers with Festucaetum. In summer polygon structures are frequently found in such areas. Perennial herbs in these areas need secure anchorage by long root systems. Small plants, such as Silene maritima var. petraea, are well adapted to survive in these habitats with a root system often longer than 1 m. Frost-induced soil movements also appear in the grazed Avenetum and were in 1972 noticed especially frequently in the harvested sample plots as well as in heavily grazed areas of this community. The trampling of the sheep in early spring has a severe damaging effect on the plant cover especially after winters with little snow.

PERMANENT SAMPLE PLOTS

The investigations on the Alvar, which aimed to show a correlation between grazing pressure and changes of vegetation, required the study of vegetational successions uninfluenced by man. Very little seems to be known about the rate of succession in different plant communities on the limestone heath whereas the directions of succession from one community to another one is fairly well known. The development of one community into another is generally resultant upon a gradual accumulation of soil. As a consequence there is a succession from lichen communities on the bare limestone rock to Festucaetum communities with fruticulous lichens and dwarf shrubs. This development is, however, most frequently interrupted by soil erosion and a regressive succession takes place. A development of the Festucaetum into the Avenetum is rare as the Avenetum is concentrated on deep soil habitats on gravelly ridges and localities on the margin of the heath. In such places there has probably been a very steady deposition of sand and gravel since early postglacial times. Succession between moist habitat communities also probably shows low dynamics. The courses of small rivulets and the sites of periodically dry shallow lakes are fairly stable. Changes of the location of moist areas probably takes place only when a drainage is changed due to the gradual formation of new fissures in the bedrock. Periodical changes of climate as a consequence of abnormally dry and wet years has only a small influence on the structure and distribution of moist communities.

As a general estimation of the dynamics in the Alvar vegetation it is suggested that changes between communities on the level of association only take place within small areas. They generally include an equal proportion of progressive and regressive successions. The reason why the rate of these successions is so little known is that a complete change from one association to another such as between the Sedetum tortelosum and the Festucaetum grassland probably requires more than half a century. A transformation of the Avenetum to a dense juniper scrub also requires more than 50 years. The development of the Sedetum tortelosum is extremely slow and may require at least 50 years, beginning from the bare rock.

Consequently the possibilities of following a successional sequence from one community to another
in permanent sample plots are not favourable on the Alvar heath. Within a short period, less than 10 years, only quantitative and qualitative changes within the limits of the natural composition of the associations will generally take place. However, it was regarded as essential to obtain knowledge about these changes. They are naturally important to questions of variations in grazing pressure and to general aspects of management connected with conservation.

The number of permanent sample plots checked once a year between 1969 and 1972 was 15 (Table 2). The climate during this period varied considerably as 1969 was a year of unusually severe drought (less than 10 mm of rain between May 25 and August 15). In 1970 the amounts of precipitation during the summer months were close to mean values, whereas 1971 and 1972 were climatically nearly normal years except for unusually high precipitation in spring and early autumn 1972).

Quantitative and qualitative changes in the plant cover of the plots can be correlated with the changing climatic situation during the four-year period. Qualitative changes are the most important ones to record.

Among the grasses there is a decreasing degree in the cover and in the presence of some species after the 1969 drought year. Dead tussocks of Festuca ovina were frequent in the summer 1970. A recolonization took place in 1971–72.

Some drought-tolerant annual species were apparently favoured during the drought year. Some perennial species did not develop in the plots in 1969. In the moist type of vegetation there was a clear increase in the number of species in 1970–72.

In the bottom layer, changes among mosses were evident. Small heptics in the Sedetum tortellosum were not present in 1969. Rhytidium rugosum and Campylopus lutescens are probably somewhat less drought-tolerant than Tortella spp., Dicranum flexicaule and Grimmia canescens. The presence of the lichens was very stable in the investigation period.

Single years of drought may cause a more pronounced dynamics among grasses than among other herbs. The productivity of such grass communities as Festucetum tortellosum and Molinietum albae are is probably more negatively influenced by drought years than the dense communities with less grasses, the Festucetum cetrarioalbae and Avenetum albae.

Extract of information from Table 2

Grasses:

Other herbs:

Lichens:
All species show a very high stability in the plots during the four-year period.

Bryophytes:
Tortella torvula, T. inclinata, Schistidium apocarpum, Tortula ruralis var. calcicola, Dicranum flexicaule are species only slightly influenced by the drought year. — Encalypta spp., Fissidens cristatus clearly unfavourably influenced by drought. F. cristatus recolonizes in 1971–71. — Campanulatetum albae colonized several plots in 1970–72. — Clevea hyalina, Rieca soroeopara, small hepatics offered bad conditions for development in a drought year. Frequently colonizing in 1970–72. — Scopidiopsis scorpioides, Sc. turgescens survived the drought year successfully. — Camptullum stellarum, Fissidens adiantoides, generally in periodically moist habitats, susceptible to drought periods probably even if these cover only parts of the period May–September.

CONCLUDING COMMENTS

The investigations into productivity and changes of plant cover on the Alvar due to high grazing pressure by sheep indicate that the relationship between grazing and of vegetational structure is very complex.

In areas heavily grazed for several years there is a suppression of grasses. Similar changes take place after repeated harvests in sample plots. Sheep-grazed areas seem to have become gradually more
### Table 2. Changes of vegetation in permanent sample plots on the Alvar heath

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<th>Year</th>
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<th>3</th>
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**Explanations**

Size of sample plots 1 m².  
Degrees of cover according to Hult-Sernander-Du Rietz.  
× denotes presence of single specimens.  
Locality: Alvar to the N. and NW. of the village of Eke- 
lunda, parish of Sandby.  
Habitats: (1) 60% of the sample plot, bare limestone rock with a few specimens of vascular plant taxa. Remaining part with thin layer of weathered gravel. Close to *Corylus avellana* shrub growing in fissures. (2) 80% limestone rock; remaining part fissures and weathered gravel, with vascular plants. (3) Weathered thin layer of gravel on rock. Narrow fissure. Colonization by vascular plants has apparently proceeded from the fissure. (4) Close to *Juniperus communis* shrub. Bare rock and gravel layer.  
(5) Thin gravel layer on the rock. Narrow fissure through the sample plot. (6) 50% bare rock, remaining part with thick gravel layer. Two fissures through the sample plot. Litter from *Corylus* shrub falls into the plot. (7) 80% bare rock, remaining part with fissure and thin gravel layer with vascular plants. (8) Close to *Corylus* shrub, source of litter to the sample plot. Thin layer of gravel. (9) Bare rock surface. (10, 11) Thin gravel layer covers the whole sample plot surface. (12, 13) Thick gravel deposits on limestone rock. (14, 15) 10 cm thick clay layer on limestone rock. (16) Borders on coarse gravel area with *Festuca vetricioides* in a mature stage of development.
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Communities (cf. Albertson 1950): (1) Sedetum tortillosum (S. t.) and Festucetum ceteriatisum (F. c.) (3) F. c. (4) S. t. and F. c. (5) F. c. (6) S. t. and transitions towards the Avenetum aloarens. (7) S. t. and F. c. (8) S. t. in transition towards the Avenetum aloarens. (9) S. t., initial stage of development. (10) F. c., initial stage of development. (11) S. t. and initial stage of F. c. (12, 13) F. c. (14, 15) Molinietum aloarens.


resistant to wearing because of the increase in herbs (except grasses) with a creeping growth-form and with a fair tolerance of grazing and trampling. A very rapid and pronounced decrease in crop thus takes place in a previously ungrazed area after the start of grazing.

The Alvar vegetation has two maxima of productivity per year, one in late June to early July and one in late August to early September. Dwarf shrubs and grasses predominate in the second maximum and other herbs in the first one. There is a decrease of productivity in July which is generally a very dry month on Öland. An increase in the amount of additional fodder provided to the animals should take place in July.

The energy content is higher in the Avenetum than in the Festucetum. In 1972 values of digestibility were 61% in the Avenetum and 50% in the Festucetum. The highest digestibility (63%) was obtained in samples from sheep grazed Avenetum, mainly as a consequence of the increased amount of herbs other than grasses there. The standing crop is higher in the Avenetum than in the Festucetum.

Ungrazed areas where the soil cover on the limestone rock is deep are invaded by shrubs, mainly juniper. Snow cover in shrub-less areas is consider-
ably less than in the juniper scrub. The presence of a sparse shrub layer in the Alvar plant communities is favourable as a protection for the vegetation during early spring. A total devastation of shrubs on the Alvar by clearing or grazing would mean serious problems of water supply to the vegetation of the heath as annual precipitation is rarely more than 400 mm. Water supply to villages in the eastern part of the island would become impaired as drainage takes place towards the E.

Clearings of shrubs in the Avenetum mean that places with loose litter and no vegetation become exposed. Grazing should be withheld until a fairly dense plant cover has colonized these places.

Frost-induced soil movements in the soil surface are especially strong in Festucetum areas. They are also strong in overgrazed Avenetum areas and thus an erosion of the plant cover by the trampling of the grazing animals is facilitated.

Succession in all Alvar plant communities is very slow. Periodical changes of climatic conditions such as single years of drought obviously influence the dynamics of the vegetation. Grasses seem to be more severely damaged by drought than are other herbs. Small drought-tolerant annual plants increase in frequency and several hepatics disappear as a consequence of a single year of drought.

The grazing pressure after a drought year should be reduced in areas with a predominance of the Festucetum in the plant cover. The number of grazing animals should be reduced (by 25–50%) and the animals should be frequently moved from one grazing area to another. Pasture rotation should, if possible, be begun during the drought year. It may be possible to return to normal grazing pressure two or three years after a drought year. If such restrictions are not carefully upheld, difficulties will eventually appear in maintaining the same grazing pressure recommended as suitable for an area (adapted to productivity) after the drought year as before.

Results of various investigations, commented on in this paper, have clearly shown that the present grazing pressure on the Alvar limestone heath cannot in general be recommended. A ewe is said to graze 2.5 kg dry matter per day, for 6 months of the year. The lambs graze an average amount of 2.5 kg dry matter for an equivalent time provided that their rate of growth is 200 g per day (i.e. the rate which is considered to be economically profitable). The average pasture land on the Alvar has been calculated to supply an annual net primary production that can provide for a maximum sheep grazing intensity of 0.65 ewes each with 1.0 lamb per hectare. To maintain an uninterrupted unaltered grazing pressure for more than 10 years it may be necessary, however, to reduce the pressure to 0.4 ewes, each with 1.0 lamb. This comparatively low number of animals per hectare may become economically profitable if an interest in quality rather than quantity is introduced in the meat production. In that way the Alvar vegetation will maintain an economic value and the valuable former landscape may also be preserved from overgrowth by shrubs, which would inevitably take place on gravelly ridges and in marginal parts if all grazing were abolished.

ACKNOWLEDGEMENTS

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REFERENCES


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