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Ernest Schockaert
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INVITED CONTRIBUTION

Linking community, evolutionary and ecosystem ecology: another perspective on plant-herbivore interactions

Michel Loreau

Laboratoire d'Ecologie, UMR 7625, Ecole Normale Supérieure, 46 rue d'Ulm, F-75230 Paris Cedex 05, France

ABSTRACT. Community ecology, evolutionary ecology and ecosystem ecology provide different perspectives on ecological systems, and have followed increasingly divergent pathways for decades. Integration of these perspectives is now critical to progress in our understanding of species interactions and ecological systems. A vivid example of the complexity generated by ecosystem processes on the very nature of species interactions is provided by the effect of material cycling on the ecology and evolution of plant-herbivore interactions. Even though they have a direct negative effect on plants through biomass consumption, herbivores can have a positive indirect effect on plant productivity through nutrient recycling. Theory shows that this indirect effect can be so strong as to prevail over the direct effect and exert effective selective pressures on the species involved provided that there is sufficient spatial heterogeneity in the system or trade-offs between traits associated with the direct and indirect effects. Thus, an exploitative interaction can turn into an ecological, and even an evolutionary, mutualism through ecosystem-level constraints. Species traits and evolution of species traits are ultimately constrained by ecosystem processes, just as ecosystem properties are constrained by the ecological and evolutionary history of interacting species. Therefore, merging the evolutionary and ecosystem perspectives, which have been increasingly separated in modern ecology, is fundamental to predicting the responses of ecological systems to environmental changes.

KEY WORDS: Plant-herbivore interactions, ecosystems, evolution, nutrient cycling, grazing optimisation, indirect mutualism.

INTRODUCTION

The vigorous growth of ecology from its origins as a distinct scientific discipline in the early years of this century has been accompanied by the creation of numerous subdisciplines. Although specialisation may be inevitable, it also creates problems. The conceptual frameworks in each area tend to become increasingly divergent over time, hampering communication across the discipline as a whole. This divergence is nowhere more apparent than between two of the major subdisciplines of ecology: population and community ecology on the one hand, and ecosystem ecology on the other hand. These two subdisciplines have grown largely independently, each having its own concepts, theories and methodologies. Ecosystem ecology is mainly concerned with the functioning of the overall system composed of biological organisms and their abiotic environment; its object is the flow of matter or energy among functional compartments; it emphasises physical and chemical constraints, and regularity and predictability at the system level. Population and community ecology is mainly concerned with the dynamics of the biological components of ecosystems; its object is biological diversity, the populations of organisms and their interactions with other populations; it emphasises biological constraints, and change and variability within systems. A third subdiscipline, evolutionary ecology, focuses on changes at long, evolutionary time-scales. It has traditionally had strong links with population and community ecology, but there has been virtually no cross-fertilisation with ecosystem ecology.

This separation of subdisciplines is understandable insofar as they partly address issues at different hierarchical levels and different spatial and temporal scales. But it is harmful insofar as it is an obstacle to their unity and mutual enrichment. In the real world, populations and communities

Corresponding author: M. Loreau, e-mail: loreau@ens.fr

do not exist in isolation; they are parts of ecosystems, and, as such, they are subjected to constraints arising from ecosystem functioning, in particular energy dissipation and nutrient cycling. These constraints can deeply alter the nature of species interactions and community properties such as food-web stability. On the other hand, ecosystems do not exist without their biological components; the latter impose their own constraints on ecosystem processes, as the disruptions generated by some biological invasions attest. In the face of the growing threat of a massive loss of biological diversity, an increasing interest is being taken in the role of biodiversity in ecosystem processes. Therefore there is today an urgent need for integration of the different perspectives (JONES & LAWTON, 1995; LOREAU, 2000). This need is felt particularly acutely at the theoretical level, where new approaches must be devised to lay conceptual bridges across subdisciplines. Theoretical studies of that kind have been few so far, but they are developing fast, and are stimulating the emergence of a new area at the interface of community, ecosystem and evolutionary ecology.

NATURAL SELECTION WITHIN ECOSYSTEMS

It is useful to start with one of the fundamental concepts of evolutionary biology, namely, natural selection. Predicting and understanding evolutionary changes and their implications require identifying the proper context of constraints within which natural selection operates.

Traditionally, evolutionary biologists considered constraints to be internal to the organisms, such as from allocations among competing needs. On this classical view, the environment is regarded as external to the organism and constant. The modern view of natural selection recognises that organisms modify and interact with their environment, which generates an organism-environment feedback in the operation of natural selection (LEWONTIN, 1983). The simplest way to obtain such a feedback is through frequency-dependent selection within a population. But there are many other ways – whether physical, chemical or biological – by which organisms modify their environment. In order to understand the full implications of the organism-environment feedback, it is further necessary to break up an organism's environment into its real physical, chemical and biological constituents and their interactions. This is what I call the 'ecosystem' view of natural selection, for an ecosystem is precisely a local system of interacting biotic and abiotic components (LOREAU, 2001). Since each organism's environment is constituted by other organisms or components, the ecosystem concept contains both the organisms and their environments. In that sense, it provides a higher-level perspective that transcends the duality between organism and environment. Recognising the ecosystem as the proper context within which natural selection, and hence evolution, operates is a major challenge for ecology today, with important implications in both basic science and more applied areas, such as conservation biology and ecosystem management.

A multitude of indirect interactions is likely to occur among organisms because of the complexity of ecosystems (PUCCIA & LEVINS, 1985; WOOTTON, 1994). These indirect effects can be weak or unpredictable (YODZIS, 1988), but some can be strong and predictable. In particular, material cycling is a key ecosystem process that drives a circular causal chain in ecosystems, thus transmitting predictable indirect ecological effects and evolutionary constraints to their component species (LOREAU, 1998). How do these constraints affect the interactions and evolution of species? Plant-herbivore interactions provide a controversial but illuminating case of this question. In what follows I focus on these interactions as one example illustrating the importance of integration of community, evolutionary and ecosystem ecology, and the fundamental enrichment that it makes possible.

GRAZING OPTIMISATION: HOW PLANTS BENEFIT FROM HERBIVORES

"Coupled transformers are presented to us in profuse abundance, wherever one species feeds on another, so that the energy sink of the one is the energy source of the other.

A compound transformer of this kind which is of very special interest is that composed of a plant species and an animal species feeding upon the former. The special virtue of this combination is as follows. The animal (catabiotic) species alone could not exist at all, since animals cannot anabolise inorganic food. The plant species alone, on the other hand, would have a very slow working cycle, because the decomposition of dead plant matter, and its reconstitution into CO_2, completing the cycle of its transformations, is very slow in the absence of animals, or at any rate very much slower than when the plant is consumed by animals and oxidized in their bodies. Thus the compound transformer (plant and animal) is very much more effective than the plant alone." (LOTKA, 1925, p. 330)

The idea that animals are detrimental to their food resources is deeply engraved on our civilisation, both culturally and economically. The need for a smooth functioning of the economy imposes a constant fight against other animal species feeding on our plant food resources, which are therefore viewed as undesirable pests from which we must protect ourselves. Ecology as a science has had to establish a more balanced view of nature. The above quote from LOTKA (1925) shows the grand view that early ecologists attempted to develop. Since then, however, even in ecology, plant-herbivore interactions have been regarded as essentially antagonistic because herbivores have a negative direct effect on plants through biomass consumption.

This traditional view has been challenged again recently by the "grazing optimisation hypothesis", which states that primary productivity, or even plant fitness, is maximised at an intermediate rate of herbivory (OWEN & WIEGERT, 1976, 1981; MCNAUGHTON, 1979; HILBERT et al., 1981). This hypothesis is supported by some empiri-

cal data, notably from the Serengeti grassland ecosystem (MACNAUGHTON, 1979). One mechanism capable of producing grazing optimisation is nutrient cycling, which mediates a positive indirect effect of herbivores on plants. Should the traditional view of antagonistic plants and herbivores be changed, can these even be mutualistic, and under what conditions? These questions, which have important consequences for both ecosystem functioning and the evolution of plant-herbivore interactions, have been at the heart of a recent controversy (e.g., SILVERTOWN, 1982; BELSKY, 1986; MCNAUGHTON, 1986; BELSKY et al., 1993; LENNARTSSON et al., 1997).

Given the ambiguity in interpretations of empirical data, we have attempted to answer these questions theoretically, using mathematical models. We have first identified the ecological conditions under which herbivores increase primary production and lead to grazing optimisation through nutrient cycling in nutrient-limited ecosystems at equilibrium (Figs 1 and 2). These conditions are two: (1) nutrient inputs (as determined by inward arrows in Fig. 1) into the ecosystem must exceed a threshold value, which is determined by the sensitivity of plant uptake rate to soil mineral nutrient, and (2) the proportion of nutrient lost along the herbivore recycling pathway must be sufficiently smaller than the proportion of nutrient lost along the plant recycling pathway (LOREAU, 1995; DE MAZANCOURT et al., 1998). Contrary to what has been assumed traditionally, nutrient turnover rates have no impacts on long-term, equilibrium primary production. These results are very general: they do not depend on the structure of the ecosystem or on the functional form of herbivore consumption. They are also potentially relevant to natural ecosystems: grazing optimisation was found to be likely for an African humid savanna (DE MAZANCOURT et al., 1999), and it can occur even if herbivory results in the replacement of a productive plant species by a less productive one (DE MAZANCOURT & LOREAU, 2000b).

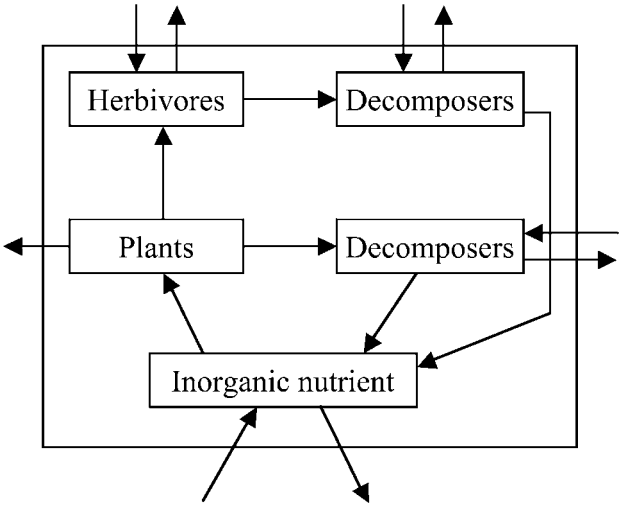

Fig. 1. – Flow diagram of the theoretical ecosystem model used to investigate the ecological conditions for grazing optimisation through recycling of a limiting nutrient. After LOREAU (1995) and DE MAZANCOURT et al. (1998).

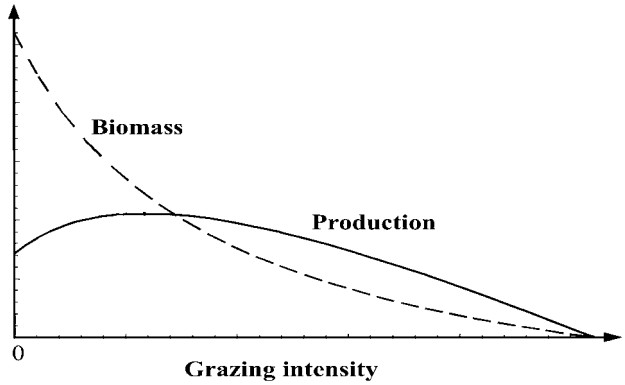

Fig. 2. – Typical grazing optimisation curve obtained for primary production at equilibrium using the model described in Fig. 1. Plant biomass, however, always decreases as grazing intensity increases. After DE MAZANCOURT et al. (1998).

THE EVOLUTIONARY PUZZLE

Does this imply that ecosystem-level constraints make the plant-herbivore interaction actually mutualistic, not antagonistic? The evolutionary consequences of grazing optimisation, and of ecological indirect interactions in general, are complex, for two main reasons. First, increased plant productivity does not necessarily translate into increased plant fitness. It is still unclear which plant traits determine fitness. If the seed production or other measures of fitness of a plant are mainly determined by its biomass, then no mutualistic interaction with herbivores is possible, because plant consumption by herbivory always decreases plant biomass (Fig. 2). On the other hand, if a plant's fitness is mainly determined by its productivity, then herbivory can increase plant fitness through increased productivity. Reality probably lies between these two extremes, and thus we may expect herbivory to increase plant fitness in some cases. Second, when it does, it is not absolute, but relative fitness that counts. If two plant types (species or genotypes) are mixed, one of them being tolerant ('mutualistic') and the other resistant ('antagonistic') to herbivory, the resistant type is expected to outcompete the tolerant type because it benefits from the positive indirect effect of increased nutrient cycling but does not suffer the negative direct effect of herbivore consumption (Fig. 3). As a result, tolerance should

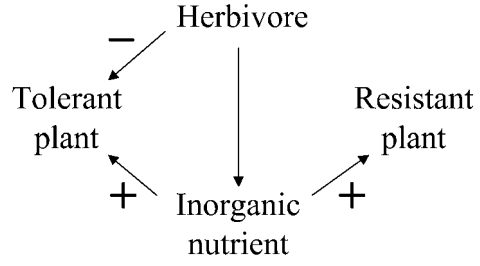

Fig. 3. – The evolutionary puzzle: if two plant types are mixed, one of them being tolerant and the other resistant to herbivory, the resistant type is expected to outcompete the tolerant type because it benefits from the positive indirect effect of increased nutrient cycling but does not suffer the negative direct effect of herbivore consumption.

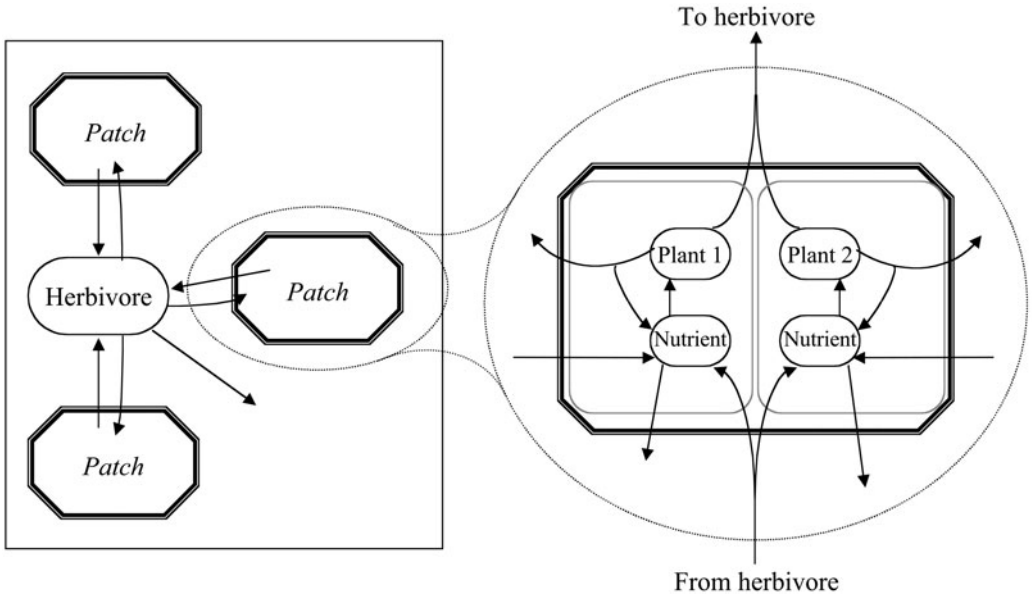

Fig. 4. – Flow diagram of the ecological model used to investigate evolution of plant palatability in a spatially heterogeneous environment. After DE MAZANCOURT & LOREAU (2000a).

not evolve even though it is indirectly beneficial. This might seem to spell the final end for the idea of any plant-herbivore indirect mutualism, indeed of any evolved indirect interaction, as some have suggested (BELSKY et al., 1993).

This conclusion is premature, however. Two factors counteract this advantage of antiherbivore defence. First, the spatial structure of the plant-herbivore system can generate spatially heterogeneous nutrient cycling (Fig. 4). If herbivores recycle nutrient in the vicinity of the grazed plants, or plants from the same type are aggregated, herbivores tend to recycle proportionally more nutrient on the plants that are grazed more heavily, thus augmenting the indirect benefit of grazing for the grazed plants. Evolution is then governed by the balance between two conflicting levels of selection, just as in the evolution of altruism (WILSON, 1980): individual selection within patches, which favours the resistant type over the tolerant one because it has a higher relative fitness, and group selection between patches, which favours patches with a higher proportion of the tolerant type because they have a higher average absolute fitness. The outcome of evolution depends on the strength of spatial aggregation and patch size: tolerance to grazing evolves provided that spatial aggregation is strong enough or patch size is small enough (Fig. 5; DE MAZANCOURT & LOREAU, 2000a).

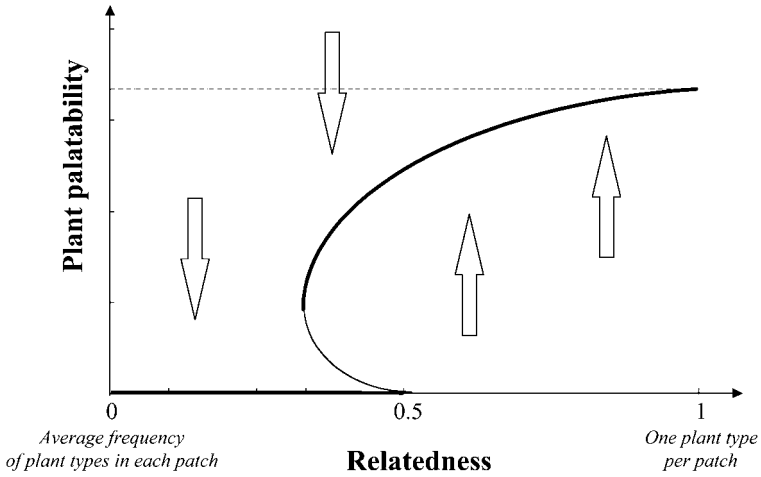

Fig. 5. – Evolutionary continuously stable strategy (CSS) of plant palatability (in bold) as a function of within-patch relatedness between plants in the model described in Fig. 4. Arrows show the direction of selection. The unpalatable plant type is always selected when within-patch relatedness is low (weak spatial heterogeneity or large patch size), but a palatable plant type can be selected when within-patch relatedness is high (strong spatial heterogeneity or small patch size). Horizontal dashed line: plant palatability that maximises primary production. After DE MAZANCOURT & LOREAU (2000a).

The second factor that counteracts the advantage of antiherbivore defence is its cost in terms of nutrient investment, which generates a trade-off in plants between defence and nutrient uptake. A theoretical study of plant adaptive dynamics (DIECKMANN, 1997) in a spatially structured model ecosystem shows that, for many ecologically plausible trade-offs, plant evolution then leads to a

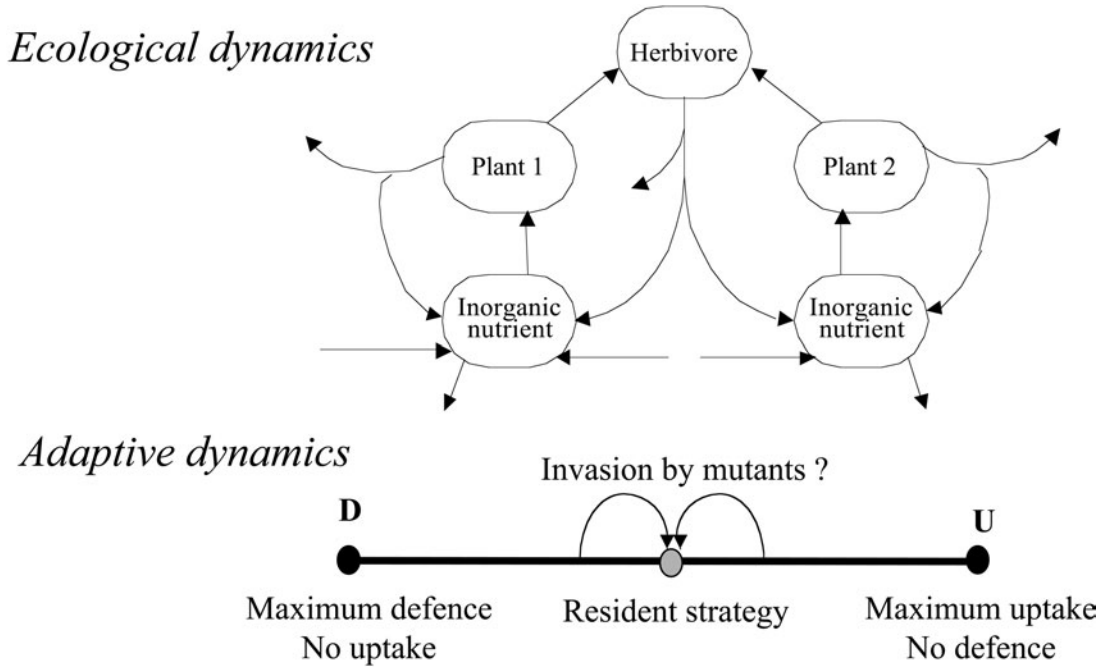

Fig. 6. – Principle of the theoretical model used to investigate evolution of plant defence subject to a trade-off with nutrient uptake (DE MAZANCOURT et al., 2001). Ecological dynamics as described in the flow diagram are assumed to unfold on a fast time-scale until an ecological equilibrium is reached. Adaptive evolutionary dynamics consists of successive invasions of resident strategies at ecological equilibrium by close mutants. Strategies are ordered along a trade-off between the two limiting cases of strategy D, in which plants invest all their resources into anti-herbivore defence, and strategy U, in which they invest all their resources into nutrient uptake.

single "continuously stable strategy" (CSS), i.e., a strategy to which evolution converges and which cannot be invaded by any other close strategy (Fig. 6). This evolutionary CSS has complex relationships with the strategies that maximise plant production or plant biomass, depending on ecosystem parameters. Because of this complexity, different ecological and evolutionary scenarios of herbivore addition or removal are possible, which highlight the ambiguity of the notion of "mutualism". It is useful to distinguish two types of mutualism: an ecological mutualism, in which each species gains a benefit from the presence of its partner in the absence of any evolutionary change, as revealed e.g. by an ecological press perturbation (BENDER et al., 1984; KREBS, 1985), and an evolutionary mutualism, in which the mutual benefit persists even after evolution has occurred (DE MAZANCOURT et al., 2001). The conditions for an evolutionary mutualism are more stringent than those for an ecological mutualism because interacting species may have evolved a mutual dependence, so that the removal of one species may have a negative impact on the other in the short term, but this negative impact may disappear after each species has had the opportunity to evolve and adapt to the new conditions created by the absence of its partner (DOUGLAS & SMITH, 1989; LAW & DIECKMANN, 1998).

When a plant's reproductive ability is determined by its productivity, herbivory is indeed capable of improving plant performance on both an ecological and an evolutionary time-scale provided that herbivore recycling efficiency be sufficiently greater than plant recycling efficiency, thus generating a plant-herbivore mutualistic interaction. Surprisingly, however, as herbivore recycling efficiency is increased, the plant-herbivore interaction becomes increasingly mutualistic (first ecologically, then evolutionarily), but at the same time plants evolve to increase their level of antiherbivore defence because they gain a higher benefit from not being consumed relative to less defended plants (Fig. 7). Thus, mutualism can go hand in hand with increased conflict between partners. Although paradoxical at first sight, such evolutionary conflicts are known in other mutualistic interactions (ANSTETT et al., 1997; LAW & DIECKMANN, 1998).

CONCLUSION

From this example, we see that species traits may evolve in counter-intuitive ways as a result of the complex indirect effects mediated by functional processes at the level of whole ecosystems. These effects may even change the very nature of species interactions, both in an ecological and in an evolutionary sense, under predictable

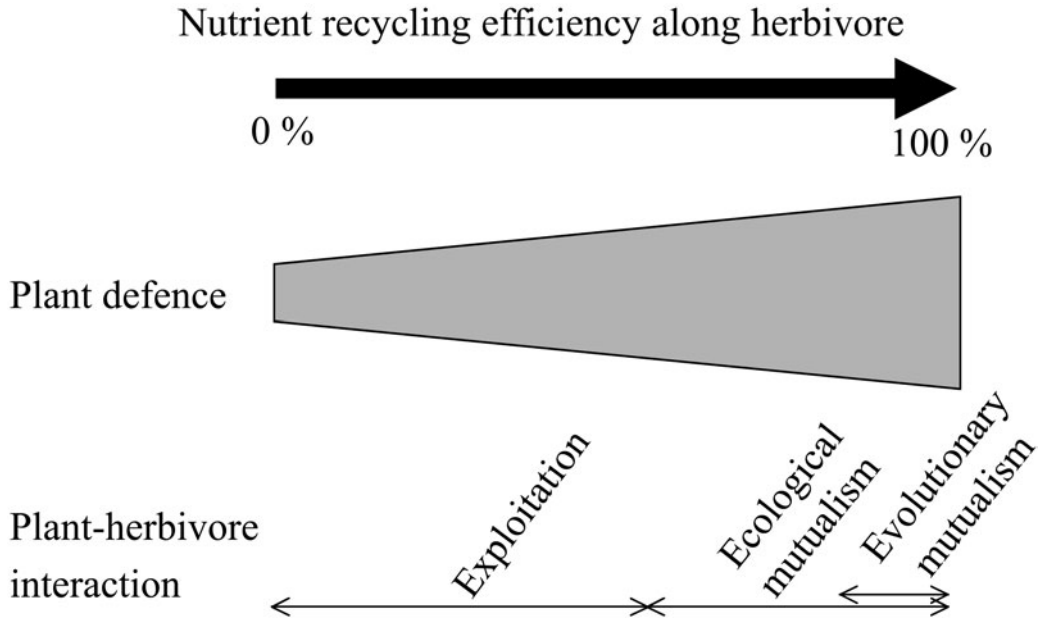

Fig. 7. – The paradox of evolution of plant antiherbivore defence: As herbivore recycling efficiency is increased, the plant–herbivore interaction becomes increasingly mutualistic (first ecologically, then evolutionarily), but plants evolve to increase their level of defence (DE MAZANCOURT et al., 2001).

conditions. Species traits and evolution of species traits are ultimately constrained by ecosystem processes, just as ecosystem properties are constrained by the ecological and evolutionary history of interacting species. Thus, merging the evolutionary, community-level and ecosystem-level perspectives, which have been increasingly separated in modern ecology, is fundamental to predict the responses of ecological systems to environmental changes, and provides mutual enrichment of the various subdisciplines. Lastly, if these theoretical considerations are correct, one implication is that conservation efforts should aim, not only to preserve species, but also to preserve the rich web of interactions in which species are imbedded in natural ecosystems, and which determine their current traits and persistence.

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Relation between Dopa Decarboxylase activity and paralytic activity in *Tenebrio molitor* and *Neobellieria bullata*

Jurgen Huybrechts, Stephen Kotanen, Arnold De Loof and Liliane Schoofs

K.U.Leuven, Laboratorium voor Vergelijkende Fysiologie en Morfologie der Dieren, Naamsestraat 59, B-3000 Leuven

ABSTRACT. Paralysins are endogenous compounds in immature insects that cause paralysis or death in adult individuals after injection into the thorax. We have proven the universal effect of paralysins by injection of paralysins from *Neobellieria bullata* into adult *Tenebrio molitor* and vice versa. The toxic effect of the tested, 30% acetonitrile fraction from whole body homogenates depends on the stage of the insect from which the extraction was made. The activity of the paralysins shows a temporal distribution with the highest level at pupation and a second, smaller effect at eclosion.

The dopa decarboxylase (DDC) activity, measured by using a radioactive labeled substrate, in developing *N. bullata* and *T. molitor*, peaks at the most important moments in the development of the insect: at pupation but especially at eclosion.

The DDC enzyme could play an important role in the toxicity of paralysins. Relating the temporal distribution of toxic activity in both species to their correlated distribution of DDC activity shows the same pattern: DDC activity increases after stages that show high paralytic activity. Injection of β-alanine-L-tyrosine (BAY, a known paralysin of *Neobellieria bullata*) into the thorax of adult flies did not induce the DDC activity. So, this could be the key to the toxic effect of BAY, because injection of BAY into the thorax of juvenile (pupae) *Tenebrio molitor* did cause an induction of DDC activity.

KEY WORDS: dopa decarboxylase, paralysins, insects.

INTRODUCTION

Paralysins recently discovered in our lab are a new class of endogenous toxic substances found in juvenile insects that cause instant paralysis or death after injection of physiological concentrations into adults (CHIOU et al., 1998a). From *Neobellieria bullata* Parker, 1916, two paralysins were purified by means of HPLC. By means of Fast Atom Bombardment Mass Spectrometry and Nuclear Magnetic Resonance spectroscopy these substances were identified as β-alanine-L-tyrosine (BAY) and 3-OH-kynurenine (3HK). The first paralysin, BAY, (CHIOU et al., 1998b) is a dipeptide with a modified N-terminal amino acid. This paralysin was known long before but in other physiological circumstances (LEVENBOOK et al., 1969). It was named sarcophagine because it was the predominant non-protein ninhydrin-positive material in fully-grown larvae of *Neobellieria* (=*Sarcophaga*) *bullata*. Sarcophagine was found to be synthesised in the fat body and to accumulate in the larval hemolymph up to the moment of the formation of the white puparium. Thereafter, its concentration drops dramatically to almost undetectable levels. The reason for this decline is that at the moment of pupariation, hydrolases from the fat body degrade the dipeptide into the amino acids β-alanine and tyrosine, which are subsequently incorporated in the cuticle, to play a role in sclerotisation (BODNARYK & LEVENBOOK, 1969; DUNN et al., 1977). This is where DDC is implicated as this enzyme is responsible for the formation of products needed in sclerotisation. The enzyme displays a high substrate specificity in arthropods (LUNAN & MITCHELL, 1969) in contradiction to the homologous enzyme in mammals (FELLMAN, 1959; CHRISTENSON et al., 1970) where the enzyme carboxylases several aromatic amino acids. CHEN & HODGETTS (1976) studied the biochemical

Corresponding author: J. Huybrechts,
e-mail: jurgen.huybrechts@ bio.kuleuven.ac.be

properties of the enzyme in *N. bullata*. N-acetyl dopamine acts as negative feed back for the DDC (FRAGOULIS & SEKERIS, 1975). We will refer to negative feed back mechanism on enzymes by down stream products in the discussion. In 1974, CHEN & HODGETTS tested the enzymatic activity in *N. bullata* in imaginal wing discs and whole body homogenates by trapping radioactive CO_2 released by the enzyme's activity. The enzyme activity peaks twice during postembryonic development: first at pupariation and second at eclosion, respectively correlated with sclerotisation of the puparium and the adult cuticle. Besides these two major peaks, there was a third smaller peak 5.5 to 6.5 days after pupariation. This peak could be responsible for the formation of the prothoracal spiracula of the pharate adult. Using a more accurate radiometric assay MARSH & WRIGHT (1980) determined the pattern of DDC expression throughout the entire development of *Drosophila*. Five defined peaks of DDC activity were recorded at embryonic hatching, the two larval moults, pupation and eclosion with the last two being the greatest. TURNBULL & HOWELLS (1980) tested the enzyme activity from *Lucilia cuprina* Wiedemann, 1830 using whole body homogenates and also found an increase of activity at pupariation and eclosion.

In this study we were looking for a temporal relation between the DDC activity and the paralytic activity during development. A relation can give us new insights in the toxicity mechanisms of paralysins.

MATERIAL AND METHODS

Animals

The fleshfly, *N. bullata* was obtained from our own breeding program at the laboratory. Individuals were kept in cages in a climate controlled room with a constant temperature of 23°C-25°C and a relative humidity of 60%-70%. There was a long day – short night cycle respectively 16h-8h. The adults were fed sugar and water until day 4. Thereafter they were also fed bovine liver, which is necessary for development of the eggs. Larvae also feed on bovine liver, and for detailed descriptions see HUYBRECHTS & DE LOOF (1977). Larvae of *Tenebrio molitor* were obtained from a local pet shop (Squamata, Herent). The larvae were kept in plastic containers in a climate controlled room with a constant temperature of 32°C and a relative humidity of 40 %. They were subjected to the same long day – short night cycle. The containers were filled (4 cm) with oatmeal and small amounts of milk powder and brewer's yeast. Pupae were collected every morning and placed in dated petri dishes. Adults were separated and also kept in dated petri dishes containing only a small amount of oatmeal.

Preparation of the solutions needed for the enzyme assay

The assay was modified after HIRUMA & RIDDIFORD (1985). Solution A: 0.5 M $NaH_2PO_4.H_2O$; solution B: 0.5 M Na_2HPO_4; homogenisation buffer: 66.0 ml A + 134 ml B (up to 1000 ml, pH: 7.1) containing 0.034 g phenylthiourea and 102.6 g sucrose. Wash buffer: 39.0 ml A + 61.0 ml B, (up to 1000 ml, pH: 7.0). Reaction buffer: 15.6 ml A + 24.4 ml B (up to 200 ml, pH: 7.0) containing 0.006 g phenylthiourea dissolved in 200 µl ethanol. L-dopa solution: 0.00986 g L-dopa dissolved in 50 ml reaction buffer. PLP solution: 0.00247 g Pyridoxal-5-L-phosphate in 50 ml reaction buffer. DEHPA solution: 1.360 ml di-(2-ethylhexyl)phosphate and 48.640 ml chloroform. Labeled L-dopa solution: 0.5 ml L-dopa solution + 0.5 ml PLP solution + 10 µl L-3,4-dihydroxyphenyl(3-^{14}C)alanine solution.

Preparation of the whole body homogenates

We respectively used two and three ml homogenisation buffer for two equivalents (one equivalent means one animal of a given developmental stage) of *N. bullata* and *T. molitor*. We prepared three samples of each stage. The homogenisation was performed with the glass homogeniser of Potter and Elvehjem (544S, 2 ml, B. Braun Melsungen AG, the pestle was driven by a boring machine: 400R electronic, AEG SBE). This was done on ice to reduce overheating from the rotating apparatus. The homogenate was centrifuged twice (15000 g, 17 min, 4°C; Beckman Optima LE-80K Ultracentrifuge) and the supernatant was stored in several portions at -80°C.

Measurement of the DDC activity

The DDC activity was measured three times per sample (3x3 measurements/stage), therefore we added 10 µl of the labeled L-dopa solution to 3 µl of sample and incubated during 30 minutes at 38°C. Three hundred µl wash buffer was added to stop the reaction (until now, steps were performed on ice). One hundred µl DEHPA solution was added and shaken well. All samples were centrifuged (11000 g, 2 minutes; Eppendorf centrifuge 5415 Belgolabo) to separate the organic layer, containing dopamine, and the aqueous layer, containing L-dopa. Two hundred µl of the aqueous layer was removed and again 300 µl of wash buffer was added, shaken and centrifuged. To 50 µl of the organic layer 4 ml of scintillation fluid was added and counted during 5 minutes. Before each measurement series a calibration of the instrument was done. We included a blank vial (3µl of sample replaced by 3µl of distilled water); a background vial (scintillation fluid only) and a positive control standard. For this standard we used white pupae of the fleshfly since this stage is easy to select and has a high DDC activity. All control pupae were collected and prepared (on the same day) for the assay using the same sample preparation as described above. The supernatant was pooled and divided into 75 Eppendorf 1.5 ml tubes and immediately stored at -80°C, until assessment of its DDC activity and subsequent scintillation count. Afterwards, that control was recalculated to the predetermined mean of the control standards

(400 dpm) and the experimental counts were then adjusted accordingly. The counts are a measure for the quantity of dopamine, so it is a measure for the enzymatic activity. Because we wanted to know the specific enzymatic activity we also determined the protein content for each sample by using the Bradford method.

For the induction experiment we used a Hamilton 100 µl syringe (2 µl per injection) for the flies. We injected anaesthetised flies (CO_2) in the thorax under the wing, more precisely under the squama. For *Tenebrio molitor*, we used glass needles pulled from capillaries (length: 75 mm, diameter 1.4-1.75) using a vertical pipette puller. The pupae were immobilised with ice and the glass needle was inserted into the thorax between the last thoracic segment and the first abdominal segment. The injected product was a sublethal dose of BAY, the paralysin discovered in *N. bullata*, 50 µg/10 µl distilled water in *N. bullata* and 50 µg/6 µl distilled water in *T. molitor*.

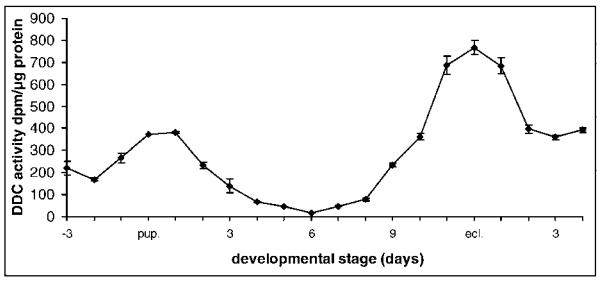
RESULTS

Determinations of the DDC activity in whole body extracts of the fleshfly and the mealworm

In *N. bullata*, the DDC activity was determined from approximately 3 days before pupation to 4 days after eclosion (Fig. 1). We noticed a slight increase of activity at pupation (371 dpm/mg protein). After this peak, the enzyme activity decreased reaching a minimum on day 6 (16 dpm/mg protein). After this temporary period of low activity the DDC activity rose again to reach a maximum at eclosion (767 dpm/mg protein). This high activity was maintained for 24 hours. After 24 hours the DDC activity dropped again and remained constant (± 400 dpm/mg protein) for the following days. In *T. molitor* we measured the DDC activity starting from approximately 3 days before pupation to 6 days after eclosion (Fig. 2). Generally the DDC activity was lower than in *N. bullata*. A peak at pupation was almost absent (65 dpm/mg protein), but was followed by a decrease, and the lowest DDC activity was reached 3 days after pupation (26 dpm/mg protein). The DDC activity peaked at eclosion (306 dpm/mg protein) and again there was a lower, but stable level of activity maintained after eclosion (100 dpm/mg protein).

Comparison between dopa decarboxylase activity and paralytic activity

This was possible as Chiou et al. (1998a) established the paralytic activity during development in the fleshfly. Thus, the corresponding data (paralytic activity and DDC activity of corresponding stages) was incorporated into one graph (Fig. 3). The same comparison was done for the mealworm. In the present study we established the paralytic profile, according to Chiou et al. (1998a). Fig. 4. shows the relation between the DDC activity and the paralytic activity in both species. An increase in toxicity (lower LD50 values) is followed by an increase of DDC activity.



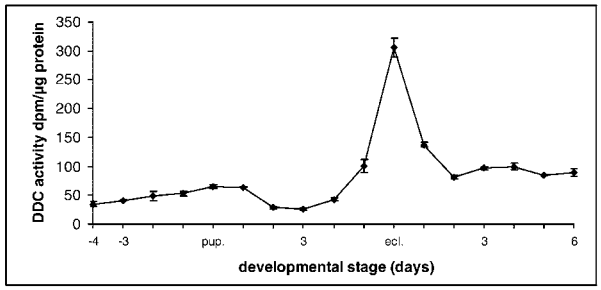


Fig. 1. – Distribution of DDC activity (presented in dpm/µg protein) during post embryonic development of *Neobellieria bullata* using whole body homogenates. Each data point represents the mean of three independent tests (three different samples of the same developmental stage) and each test concludes three different counts of the same sample. Vertical lines represents standard error.

Fig. 2. – Distribution of DDC activity (presented in dpm/µg protein) during post embryonic development of *Tenebrio molitor* using whole body homogenates. Each data point represents the mean of three independent tests (this is three different samples of the same developmental stage), each test concludes three different counts of the same sample. Vertical lines represents standard error.

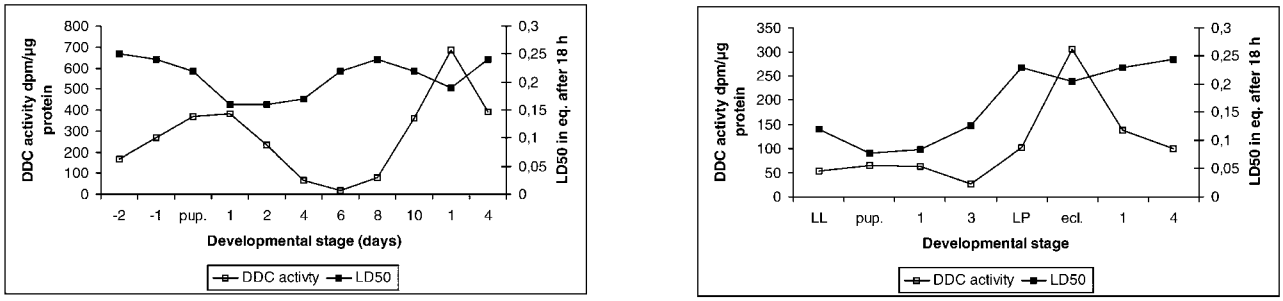


Fig. 3. – Relation between DDC activity and paralytic activity in developing *Neobellieria bullata*. DDC data is copied from Fig. 1. The LD50 values are represented in equivalents of the injected 30% ACN/0.1% TFA extract (extracts from developing *Neobellieria bullata* into adult *Neobellieria bullata*). Each data point represents the mean of three independent tests, and every test included ten flies. The standard deviation is never greater than 10 %. Injection of controls with distilled water had no effect. The LD50 values are obtained from Chiou et al. (1998a).

Fig. 4. – Relation between DDC activity and paralytic activity in developing *Tenebrio molitor*. DDC data are copied from Fig. 2. The LD50 values are represented in equivalents of the injected 30% ACN/0.1% TFA extract (extracts from developing *Tenebrio molitor* into adult *Neobellieria bullata*). Each data point represents the mean of three independent tests, and every test included ten flies. The standard deviation is never greater than 10%. Injection of controls with distilled water had no effect. The LD50 values are obtained from unpublished results.

Induction experiments

Fig. 5 shows the DDC activity in whole body homogenates of adult flies injected with 50 µg BAY into the thorax. All flies were injected at day 2 (this is 2 days after eclosion). One day after injection, there was no difference in activity between our experimental conditions (50 µg BAY dissolved in 10 µl distilled water) and the two controls (untreated flies and flies that were injected with 10 µl distilled water). Two days after the injection, the DDC activity of flies under the experimental condition decreased but remained close to both controls. Fig. 6 shows the DDC activity in whole body extracts of pupae of *T. molitor* injected with 50 µg BAY into the thorax. All pupae were injected on day 1 (this is 1 day after pupation). One day after injection, there was already a distinct difference in activity between our experimental condition (50 µg BAY dissolved in 6 µl distilled water) and both our controls (pupae without injection, pupae injected with an equal volume of solvent). The DDC activity had more than doubled from the first day values and it continued to rise on the second day while the controls further decreased.

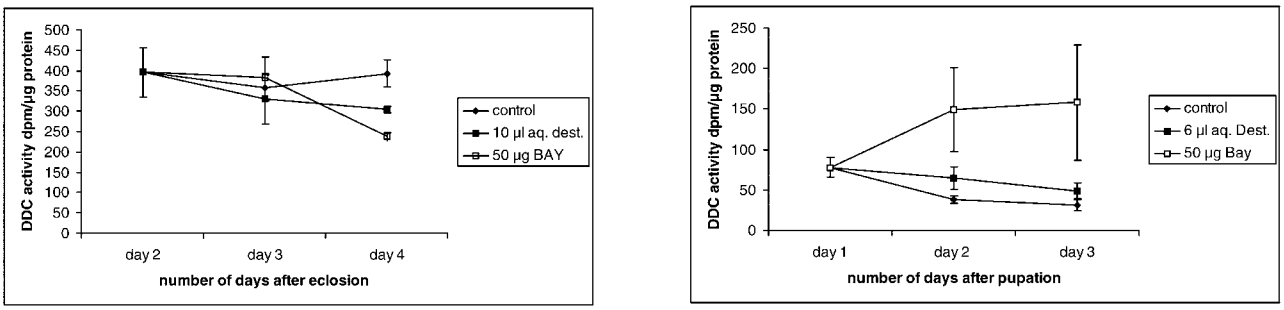


Fig. 5. – Influence of BAY on the DDC activity in adult *Neobellieria bullata*. Controls were left untreated or injected with 10 µl distilled water. The experimental condition was 50 µg BAY dissolved in 10 µl distilled water. The determination and calculation of DDC activity were the same as described in Fig. 1.

Fig. 6. – Influence of BAY on the DDC activity in juvenile *Tenebrio molitor*. Controls were left untreated or injected with 6 µl distilled water. The experimental condition was 50 µg BAY dissolved in 6 µl distilled water. The determination and calculation of DDC activity were the same as described in Fig. 1.

DISCUSSION

Dopa decarboxylase assay (Figs 1-2)

As previously stated, DDC plays a very important role in the metabolism of insects. It is necessary for sclerotisation and this is reflected in the high activity at pupation and eclosion in both species. In *T. molitor*, we see a slight increase of DDC activity in whole body extracts at pupation but the highest activity we measured at eclosion (six times higher than at pupation). These are in relation to the hardening and colouring of the pupal and adult cuticle. The pupae (pupa libra) stay relatively soft and light coloured whereas adult beetles develop a very hard and dark exoskeleton over a time period of two days. The overall activity compared to *N. bullata* is four times lower, which may be related to the intensity of sclerotisation.

In *N. bullata*, there is significant increase in enzymatic activity at pupation in contrast with *T. molitor*. This high activity is correlated with the formation of the puparium. This puparium is the larval skin that turns very hard and very dark to protect the developing insect inside. The highest DDC activity, at eclosion, is correlated with the formation of the adult cuticle. The fact that the activity starts to rise before eclosion is related to the mobilisation of products needed for sclerotisation and pigmentation. A remarkable fact is that CHEN & HODGETTS (1974) had measured a third but small activity peak at the moment we measured the lowest DDC activity (day 6). The temporal relation between dopa decarboxylase activity and paralytic activity is remarkably similar in both species (Figs 3-4). It seems that an increase in toxicity is followed by an increase of enzymatic activity. During the transition larval – pupal we see a gradual increase of toxicity associated with a gradual increase of enzymatic activity. After pupation we see the inverse effect.

Experimental data from the injection experiments suggest that we are able to induce DDC activity in juvenile *T. molitor* by injection of BAY (BAY was never identified in *T. molitor* as a paralysin, and we assume that there is a similar small peptide for storage of tyrosine in the beetle.). This is not possible in adult *N. bullata*. Here, we may find the key to the toxicity of BAY in adult flies. Consider the natural pathway from BAY to dopamine in the opposite direction (Fig. 7). Accumulation of L-dopa then leads to an accumulation of L-tyrosine because of a negative feedback of tyrosine-hydroxylase. The accumulation of L-tyrosine inhibits the dipeptidase responsible for the mobilisation of BAY. The BAY itself accumulates to toxic concentrations.

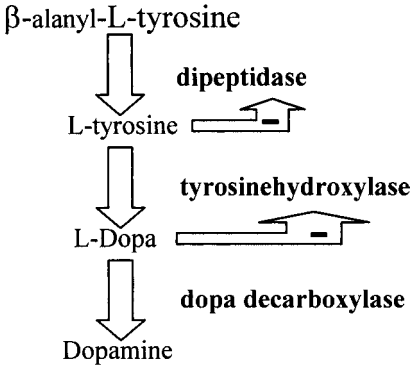


Fig. 7. – The followed pathway of BAY to dopamine and the involved enzymes (bold). Straight arrows indicate the formation of products, bent arrows with "-" indicate the negative feedback. If DDC is induced as in juveniles, dopamine will be formed. If DDC is not induced as in adults, L-dopa accumulates and is responsible for the start of the negative feed back.

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Ultrastructural aspects of two sting glands in social Hymenoptera

Eric Schoeters and Johan Billen

**Zoological Institute, University of Leuven,
Naamsestraat 59, B-3000 Leuven (Belgium)**

ABSTRACT. Exocrine glands associated with the sting apparatus are widespread in social Hymenoptera. Well-known are the venom gland and the Dufour gland. However, some other glands have been reported in literature. One example is the 'sting gland' in the region of the triangular plate part of the sting apparatus. This gland belongs to the secretory unit type 3. The duct cells open through an intersegmental membrane near the base of the triangular plate. In the present study, this site of duct openings is discussed in the perspective of its association with the oviduct and not with the sting sensu stricto. The postulated involvement of the gland in reproduction gains in importance, because obvious differences concerning glandular size were found between queens and workers. This tendency is observed for some ants, for bumblebees and for social wasps.

In general, this triangular plate gland seems to metabolize lipids. The fact that some queens possess extremely developed glands most probably has little to do with lubricant production needed for facilitating the act of stinging, as is sometimes suggested in literature, since usually workers are more likely to sting throughout their lives, and queens will especially sting at the more critical stage of colony foundation.

A second gland, the quadrate plate gland was found near the quadrate plate of the sting in bumblebees. Ultrastructural characteristics of both glands are described.

KEY WORDS: exocrine glands, triangular plate gland, quadrate plate gland, social insects.

INTRODUCTION

Social insects are known to be well provided with exocrine glands (BILLEN, 1993; BILLEN & MORGAN, 1998; HÖLLDOBLER & WILSON, 1990). Most of the glands play an essential role in the social life of these insects. Even today, new glands are being discovered. The objectives of the present study are 1) to provide more detailed data concerning a gland that was once mentioned briefly in literature, the triangular plate gland (or sting gland according to ROBERTSON, 1968) and 2) to describe a novel gland in bumblebees, the quadrate plate gland.

Usually, concerning sting glands, two particular structures are of interest: the venom gland and the Dufour gland. The venom gland is involved in venom production in stinging species, but can have various other functions, such as the production of pheromones. The Dufour gland often produces hydrocarbon mixtures with various functions (BILLEN & MORGAN, 1998).

However, the glands dealt with in the present study are rather intersegmental glands that are more indirectly associated with the sting. JESSEN & MASCHWITZ (1983) have provided a detailed overview of abdominal glands in *Pachycondyla tridentata* (F. Smith, 1858), a ponerine ant. Most of the glands mentioned in their work are intersegmentally located. In that study, an account was also given of the glands associated with the sting apparatus itself (triangular plate gland, quadrate plate gland, spiracular plate gland, sting sheath gland). However, only light microscopical data are available.

MATERIAL AND METHODS

Foraging workers and queens of *Myrmecia pyriformis* F. Smith, 1858, *Diacamma* sp., *Odontomachus rixosus* F. Smith, 1857, *Pachycondyla* sp., *Bombus terrestris* (Linnaeus, 1758) and *Vespa crabro* Linnaeus, 1758 were dissected in insect Ringer solution (Jolly) and then fixed in glutaraldehyde. A first group of samples was dehy-

Corresponding author: E. Schoeters,
e-mail: eric.schoeters@bio. kuleuven.ac.be

drated in a graded ethanol series. After dehydration, samples were put into LRWhite resin (three times rinsed). Consequently embedding in LRWhite was performed (at 50 °C). LRWhite embedded material was then processed for histochemical staining (for protein) by means of Coomassie blue.

Sting glands were fixed during 2-20 hours in 2% glutaraldehyde (4 °C, pH 7.3 and buffered with 0.05 M sodium cacodylate). Postfixation in a buffered osmium tetroxide solution (1 hour) was followed by dehydration in an acetone series and embedding in Araldite.

Semi-thin sections (1 µm thickness) for light microscopy were made with a Reichert OmU2 ultramicrotome and stained with methylene blue and thionin.

Thin sections, made with a Reichert Ultracut E microtome, were stained with uranyl acetate and lead citrate in an LKB 2168 Ultrostainer, and examined in a Zeiss EM 900 electron microscope.

Samples for SEM analysis were critical point dried in a Balzers CPD 030 critical point drying device, after complete dehydration in formaldehyde dimethyl acetal (dimethoxymethane or methylal). They were coated with gold and viewed in a Philips SEM 515 microscope.

RESULTS

The triangular plate gland

A series of species belonging to different families of social insects were screened to check for the presence and development of their sting glands. Particular attention was paid to the occurrence and development of the triangular plate gland (or sting gland, following ROBERTSON, 1968). Each triangular plate gland consists of a cluster of secretory units, belonging to type 3 according to NOIROT & QUENNEDEY (1974) and QUENNEDEY (1998). This means that each secretory cell is provided with its own duct cell.

In the species studied, sting glands are usually well developed towards the region of the triangular plate (Fig. 1), where these glands are attached via their ducts to the intersegmental membrane. However, the position is not always the same, because of the glandular cluster size. In bumblebees, for example, and more particularly in their queens, a more ventral (with the sting as reference) location is found. Bumblebee queens are among the most interesting insects to demonstrate the position and development of the triangular plate gland, as is illustrated in Figs 2 and 3. From these figures, it is clear that, notwithstanding the fact that from a lateral view the gland seems to be located opposite to the triangular plate, it is also clearly associated with the posterior end of the oviduct, and hence masking its relevant position.

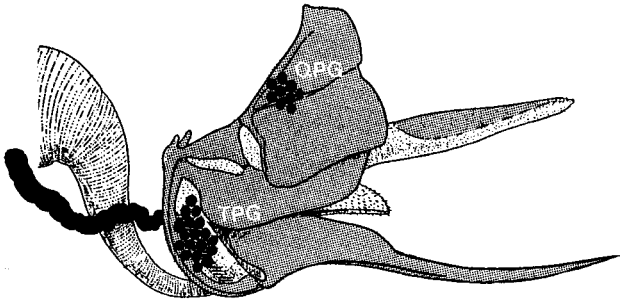

Fig. 1. – Schematical representation of two glands associated with the sting in bumblebees. Lateral view of sting apparatus; at the left are the Dufour gland (black) and the duct of the venom gland; QPG=Quadrate plate gland; TPG=Triangular plate gland.

When the sting is moved backwards to allow egg-laying, however, the ducts most likely will discharge their secretion more ventrally into the posterior part of the central oviduct.

The ultrastructural characteristics of the gland include occurrence of smooth endoplasmic reticulum, the end apparatus (Fig. 5), lipid inclusions of varying diameter, electron-dense inclusions and multilamellar bodies. The last were conspicuously found in association with lipid droplets in the triangular plate gland of *Odontomachus rixosus* queens (Figs 5 and 6) and are known from other studies to be involved in the actual secretion process. Lamellar bodies, similar to those found in vertebrate surfactant producing cells, were also found near the end apparatus (Fig. 5). *O. rixosus* workers, however, do not seem to have this gland. In contrast, queens possess a glandular cluster of about 300 µm in diameter, with glandular cells having a diameter of approximately 40 µm.

Legends to the figures (see opposite page)

Fig. 2. – Scanning electron micrograph of the triangular plate gland in a *Bombus terrestris* queen. Lateral view of sting apparatus. The arrow indicates the position of the gland near a cluster of fat cells. DG=Dufour gland; St=sting; TPG=triangular plate gland. Scale bar 1 mm.

Fig. 3. – Scanning electron micrograph of the triangular plate gland in a *B. terrestris* queen. Ventral view of sting apparatus, showing both glands. OD= oviduct; St= sting. Scale bar 1 mm.

Fig. 4. – Semi-thin section of LRWhite-embedded triangular plate gland in a *Myrmecia pyriformis* worker. The dark staining end apparatus after application of Coomassie Blue for protein in each cell is clearly visible (arrows). DC=duct cells. Scale bar 10 µm.

Fig. 5. – Electron micrograph of the triangular plate gland in an *Odontomachus rixosus* queen, showing end apparatus (EA) with slight microvillar distortion. LB=lamellar bodies; mv=microvilli. Scale bar 1 µm.

Fig. 6. – Electron micrograph of the triangular plate gland in an *O. rixosus* queen, showing secretory vesicles of mixed composition. li=lipid material; mt=mitochondria. Scale bar 1 µm.

Fig. 7. – Electron micrograph of the quadrate plate gland in a *Bombus terrestris* worker, showing end apparatus without microvillar distortion. lu=lumen of the end apparatus. Scale bar 1 µm.

Fig. 8. – Electron micrograph of the quadrate plate gland in a *B. terrestris* worker, showing inclusions of mixed composition. my=myeloid secretion. Scale bar 1 µm.


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TPG
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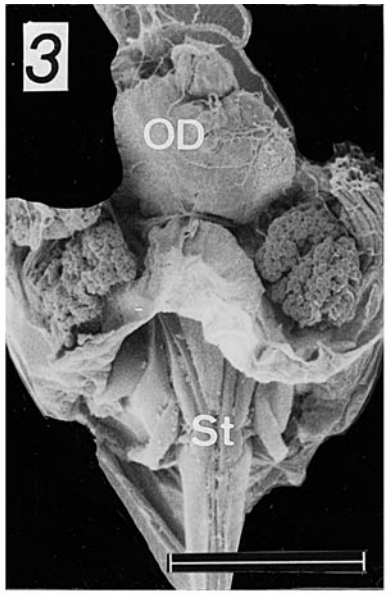
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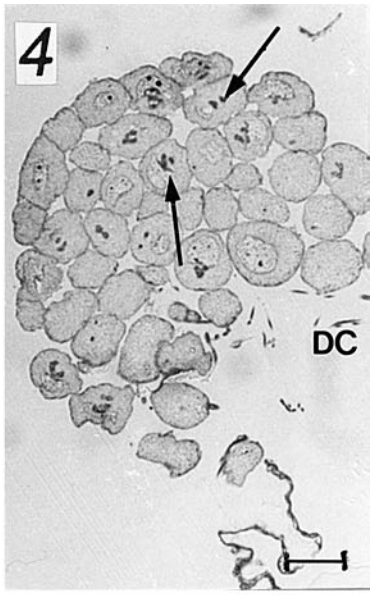
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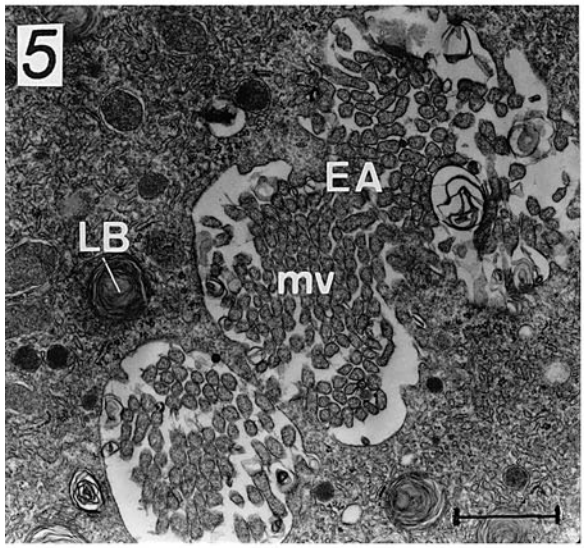
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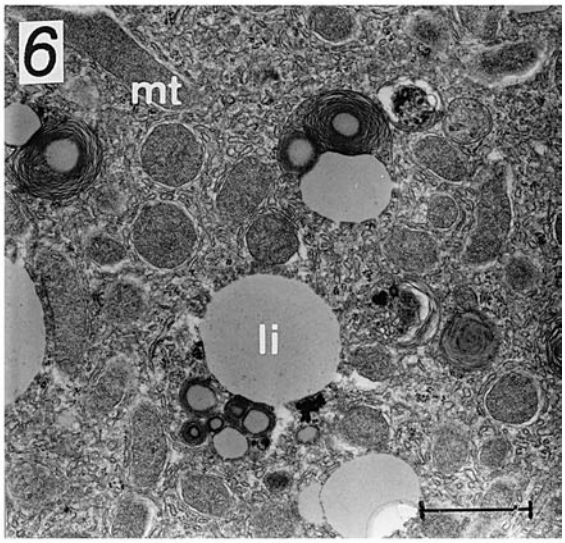
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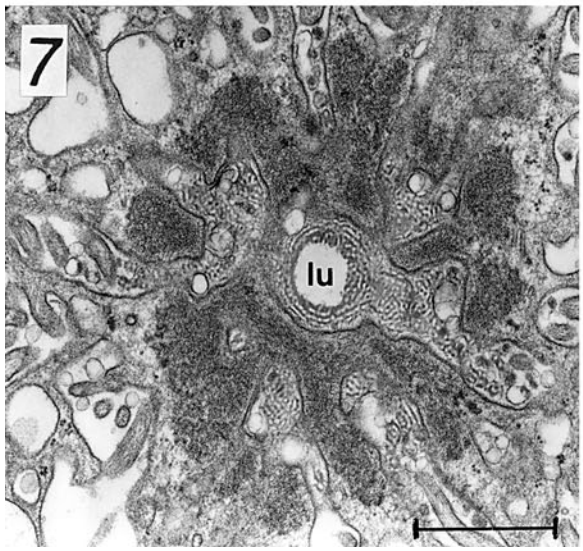
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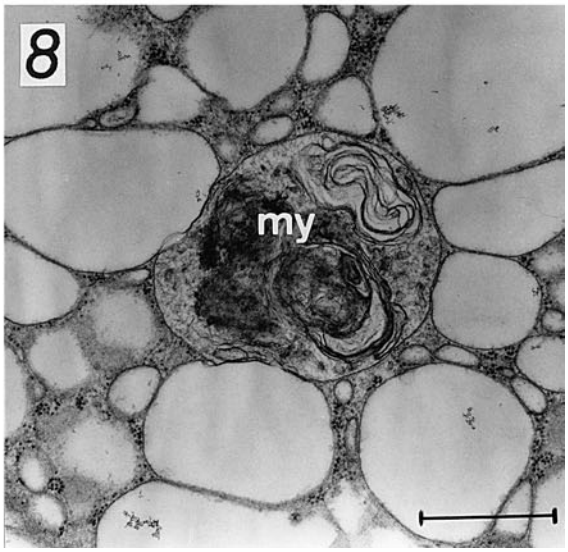
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my

The triangular plate gland thus has ultrastructural characteristics of a gland producing lipoid substances. However, histochemical investigation for protein in *Myrmecia pyriformis* has revealed the presence of proteinaceous material in the lumen of the end apparatus of each secretory cell (Fig. 4). Additional histochemical analyses have shown that a minor fraction of the secretion contains polysaccharides.

The quadrate plate gland in *Bombus terrestris*

In bumblebees we found a glandular cluster (see Fig. 1), near the quadrate plates associated with the sting.

Each cluster consists of secretory units. The ducts of these units discharge their secretion through the intersegmental membrane between the quadrate plate and the spiracular plate. The quadrate plate gland of the bumblebee *Bombus terrestris* is fairly similar in ultrastructure to the triangular plate gland. Sections through the end apparatus can be encountered (Fig. 7), as well as inclusions consisting of variable contents (Fig. 8), which can be designated as myeloid secretion.

DISCUSSION

The present study is the first to provide more accurate data concerning the morphology, the ultrastructure and the histochemistry of the triangular plate gland in social Hymenoptera. The only available literature data dealing with this gland are simple descriptions for some species on the occurrence or absence of the gland, without further specification of cellular morphology and/or ultrastructure (ROBERTSON, 1968). We have tried to fill this gap and have shown that, at least in bumblebees, even another 'sting gland' occurs near the quadrate plate. In earlier literature on honeybee glands by SNODGRASS (1956), it was already suggested that this glandular mass of unicellular glands producing their secretion to the outside of the quadrate plates of the sting in *Apis mellifera*, might lubricate the shaft of the sting when venom is ejected. However, it has been shown by GHENT & GARY (1962) that an attractant is produced, which stimulates other bees to continue their attacks at the initial stinging site. This assumption remains controversial, because the gland as we found it in bumblebees is not very likely to serve a similar function, knowing that bumblebees usually are far less aggressive.

In general, the triangular plate gland seems to metabolize lipids, but apparently lipids are not the only constituents of the secretion, since histochemical staining with Coomassie blue has revealed the presence of protein in the end apparatus of each secretory unit (in bulldog ants of the genus *Myrmecia*). Even sugars are present.

ATTYGALLE et al. (1996) screened a set of small dermal complex glands in the large ponerine ant *Pachycondyla tridentata* (F. Smith, 1858), a glandular system which consists of groups of gland cells located dorsally, ventrally, dorsoventrally, dorsolaterally and ventrolaterally in the intersegmental membranes between all gaster segments, and also near the sting apparatus. For the single species mentioned by these authors, oily substances were found and hence a lack of pheromone function and antibiotic effects was shown together with more evidence for a lubricative function. Significant amounts of linoleic acid and palmitic acid, together with trace amounts of other fatty acids and corresponding methyl esters have been demonstrated. According to the same authors, similar glands can be expected to be found in association with the sting apparatus as the sclerites of the sting apparatus derive from abdominal sclerites. However, our observations on *Odontomachus* ants, also belonging to the same subfamily as *P. tridentata* investigated by ATTYGALLE et al. (1996), suggest a shift in glandular function, since the lubricative gland seems absent in workers but is very obviously present in queens. It is indeed important to bear in mind that functional specialization within one particular category of glandular structures can occur, as is known for other sting glands as there are venom glands (with production of protein, alkaloids, pyrazines, formic acid, etc.) and Dufour glands.

In bumblebees and ants, cautious interpretation is recommended, because bumblebee and ant queens might need a powerful sting especially during the critical time of colony foundation, but this argument does not explain why e.g. in *Odontomachus rixosus*, the queen has such a large gland whereas the workers apparently lack it. *Odontomachus* workers frequently need their sting for predator-prey interactions and they do not have the gland, so the argument of a lubricative function is not valuable in this case. The fact that in *Odontomachus* ants, queens possess highly developed glands most probably has little to do with lubricant production needed for facilitating the act of stinging, as is sometimes suggested in literature, since usually workers are also very likely to sting. It would then be very unlikely to find an extremely large gland in the queen. In workers of *O. rixosus* we failed to trace the gland, so the difference between the castes is even more pronounced. In various social insect groups, workers are more likely to sting throughout their life, when facing prey and/or possible predators, whereas queens will sting especially during the critical time of colony foundation.

The observation that protein is present in the secretion is important in the light of the findings of CASSIER & LENSKY (1994) for the Nasanov gland of the honeybee. These authors pointed out that the protein present in the secreted material would enhance the effect of the biological activity of the released chemicals. Usually, the problem in analysing secretions by means of one particular technique, e.g. during the search for pheromones by gas chromatography, is that often other constituents of the secretion are being overlooked. An example from literature is known for tergal glands of cockroaches (QUENNEDEY & BROSSUT, 1975). These authors found that protein is indeed present in the gland cells, but the direct

function of these molecules has not been elucidated. Because of its very obvious presence, the protein is not likely to be a structural element within the endocuticula of the gland end apparatus, at least for *Myrmecia*. The histochemical results in this study clearly point in the same direction, i.e. that a more cautious interpretation should be recommended. In general, proteins, often in combination with lipids or glucids, are common in secretions, but are generally underestimated because of the analytical techniques used in one particular research field.

The precise site where the ducts of the secretory units open can be situated in the intersegmental membrane near the triangular plate, but if the insect extrudes its sting, the ducts will open more ventrally into the cavity of the central oviduct. This is the controversial point we would like to emphasize in the present study. From our semi-thin sections and dissected stings, we therefore propose not to exclude a role in reproduction for the triangular plate gland. When the female starts egg-laying, the egg will pass directly underneath the sting base, and hence contact of the egg with glandular secretion is a possibility. One could imagine some kind of egg-marking. The myeloid secretion found in the glands studied, is also known from mandibular glands of the cockroach *Blaberus craniifer* (Quennedey & Brossut, 1975).

We have checked the occurrence of the triangular plate gland in several species of *Myrmecia* (Myrmeciinae), in several representatives of the Ponerinae, such as *Pachycondyla*, *Odontomachus* and *Diacamma* species, in social wasps and in bumblebees. Its widespread occurrence suggests a fundamental function in the biology of these insects.

Summarizing our findings, we can conclude that: 1) the triangular plate is present in more species than previously reported, 2) its secretion is lipoidal, but not uniquely. Part of the secretion is protein, 3) the gland shows, at least in some species, a clear queen-worker dimorphism, 4) the triangular plate gland is also present in other groups of social insects, such as bumblebees, 5) the triangular plate in bumblebees is also closely associated with the end of the oviduct, which probably implies a reproductive function and 6) other intersegmental glands such as the quadrate plate gland, are present in bumblebees.

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Developmental stability as the primary function of the pigmentation patterns in bivalve shells?

Vincent Bauchau

Rue R. Christiaens, 12, B-1160 Bruxelles

ABSTRACT. Most bivalve shells (and other seashells) display complex and highly diverse pigmentation patterns. Crypsis, however, can only explain the presence of complex pigmentation in a few specialised species. Many species live under the sediment and/or their shells are covered by an opaque periostracum. The primary function of the pigmentation patterns, if any, remains a puzzle. I propose here that the pigmentation is intimately associated with the regulation of the growth of the shell to achieve developmental stability. More specifically, I suggest that the pigmentation pattern creates heterogeneity along the shell margin (and incidentally on the rest of the shell) in order to allow the (richly innervated) mantle to position and regulate its shell-growing activity.

KEY WORDS: pigmentation pattern, marine molluscs, sea shells, development, asymmetry, shell growth.

INTRODUCTION

Molluscan shells, particularly seashells, often display eye-catching pigmentation patterns whose sheer diversity and beauty have attracted the attention of collectors and scientists alike. Often the patterns have a striking and complex geometry reminiscent of patterns obtained by mathematical models such as fractals, cellular automata, and Turing reaction-diffusion models. Indeed intensive work on mathematical models has shown how pigmentation patterns can be generated and what allows for their considerable variety (reviewed MEINHARDT, 1995). However the primary function of the pigmentation pattern, if any, is not established and remains an evolutionary puzzle. Secondary functions, such as crypsis, have been occasionally described but are only relevant for a very small number of species.

Here I will review the main known data about pigmentation patterns in bivalve shells and other seashells, and propose a new hypothesis for their primary function. I will suggest that the pigmentation pattern is part of the mechanism for developmental stability, i.e. the mechanism that allows the mollusc to grow a shell corresponding to the optimal shape (e.g. in equivalve bivalves the two valves must be symmetrical). The neural system of the mantle would be involved in sensing the current pigmentation pattern and use it to position its activity, i.e. deposit new pigmentation and new shell material in a finely regulated way. Some possible means of testing this speculative hypothesis will be also suggested.

Corresponding author: V. Bauchau,
e-mail: vincent.bauchau@worldonline.be

SOME EARLIER IDEAS

The present paper will concentrate on bivalves, although most of what will be said here is also valid for other molluscs. The reasons to concentrate on bivalves are as follows. Firstly, bivalves, at least in some families like the Veneridae, exhibit pigmentation patterns whose complexity and diversity rival those in other groups of seashells known for their pigmentation (e.g. gastropods: Conidae, Olividae). Secondly, the pigmentation patterns in bivalves exist in two copies, one on each valve. A crucial observation, which will be discussed further below, is that, at least in some species, there is mirror-symmetry between the pigmentation patterns on the two valves. Finally, because of their abundance, diversity and economic interest, bivalves have received much attention in ecology, population genetics, palaeontology, environmental monitoring and commercial management of stocks. Thus there is a rich body of data on the biology of bivalves, especially on growth and shell formation.

Many bivalves spend the majority of their lives buried in the sediment. Often the shell is completely covered by an opaque periostracum and/or sessile organisms. Furthermore, bivalves have no eyes (although Pectinidae

have well-developed ocelli). Therefore, contrary to other colourful animals (birds, insects), pigmentation patterns in (most) bivalves cannot function as a signal. A very small number of species that live on the seafloor or other substrate (rocks) may be cryptic but the evidence is limited (Cox et al., 1969; Cain, 1988). Another case of adaptive protection against predation would be the massive colour polymorphism in some species (mostly in the genus *Donax,* possibly *Macoma)*, where predation rate would be decreased by the inability of the predators to develop a search image for all possible morphs (apostatic selection; Moment, 1962; Allen, 1988; Cain, 1988). In any case the function of pigmentation pattern as a protection from predation is a secondary function, limited to only very few species. In most species the pigmentation pattern is not cryptic, and there are only few species where the intraspecific variability is sufficient for apostatic selection. The role of the pigmentation pattern in thermoregulation is another possibility, supported by the observations, at least in *Mytilus* species (Mitton, 1977), but again this secondary function can only be adaptive in a few species.

Thus many authors have accepted the idea that pigmentation patterns have no (primary) function at all, and that the high polymorphism in pigmentation pattern, within as well as between species, is a sign that natural selection is relaxed (e.g. Seilacher, 1972; Ermentrout et al., 1986; Meinhardt, 1995). The idea that pigmentation patterns are not functional, however, seems at odds with their complexity and potential cost of production. Hypotheses for primary functions have been suggested, but none can be considered as adequate. It has been suggested that pigments are waste products of metabolism, secreted in the shell as a means of disposal. This popular idea seems to originate from Comfort (1951), who stated that in most of the primitive molluscs, shell pigments are almost certainly secreted in the shell as a means of disposal, being either derived from the diet or from unmanageable metabolic residues; no other details or references were given. The hypothesis of pigmentation as waste disposal does not explain why the pigmentation is often so complex. Furthermore, molluscs have a well developed excretory system and it is unclear why some of the metabolic product should be deposited in the shell instead. In some species, the concentration of pigment in the shell correlates with the concentration of chlorophyll (from which the pigment is derived) in the food available in the habitat (Underwood & Creese, 1976; see also Leighton & Boolootian, 1963). However, even if shell pigments are derived from the food or waste product, their primary function is not necessarily waste. On the contrary, their presence on the shell (as opposed to release into the water) implies that they have some other function. This is in line with the general trend within animals, which is to obtain pigments from their diet, whatever the function of their pigmentation pattern. Another suggestion for the function of pigmentation is that it strengthens the shell (Cain (1988), actually referring to the coloration *inside* the shells). Some pigments in the shell are indeed intimately associated with conchiolin, the organic matrix of the shell material (Comfort, 1951). To my knowledge, however, there is no evidence that pigmentation enhances shell strength.

The structure of this paper is as follows: in the next section, I briefly review what is known about shell growth and about the physiology of pigmentation, the two being developmentally (and, I suggest here, functionally) related. Then I review the work on mathematical models of pigmentation patterns. These models are important because they successfully predict the observed patterns and their variation, and, in the case of bivalves, they suggest that mirror-symmetry between the two valves is difficult to achieve. I then explain my hypothesis and discuss how it relates to the known data. Experimental tests for the hypothesis are also suggested.

WHAT IS KNOWN?

Shell growth in bivalves

The shell consists of calcium carbonates and an organic matrix, both deposited by the outer layer of the mantle, a large sheet of tissue consisting of two lobes, one lining each valve. The two lobes are connected dorsally. A nerve, called the circumpallial nerve, runs along and parallel to the mantle edge, which is rich in sensory organs, including receptors that may be involved in the regulation of the shell formation (Saleuddin, 1979:72). The mantle attaches to the shell along the pallial line, which runs parallel and close to the shell margin. Growth of the shell takes place when the bivalve is respiring and feeding: the valves are open and the mantle edge is protruded. The inner surface of the shell is covered by layers of nacre or other microstructures deposited by the entire outer surface of the mantle. This thickening of the shell will not be considered here; in this paper, growth of the shell refers to the growth in size, at the margin. The above description of shell growth is schematic and there are some variations but they need not be considered here.

The growth of the shell is cyclic, as evidenced by external markings (growth lines with seasonal or annual periodicity, i.e. related to large environmental changes) and internal microgrowth increments (visible under the microscope, with a periodicity of the order of a day). The latter cycle may be caused by periodic valve closure, e.g. at low tides for species living in the intertidal zone (Lutz & Rhoads, 1980) but there is also evidence that it is uncoupled to environmental variation, suggesting some endogenous mechanism (Berard et al., 1992).

Pigmentation

The pigmentation is deposited on the shell surface by the mantle edge, during shell growth. Thus, the patterning

of the shells is a graphical representation, in time, of secretory activity along a line of cells, the mantle edge (COMFORT, 1951). Pigmentation patterns usually involve one or two colours (other than white), at least under ambient lighting. Pigments include melanins, pyrroles and porphyrins (COMFORT, 1951) but they have not all been identified.

Patterns of pigmentation on seashells vary from simple to extremely complex. Some shells appear completely white (the natural colour of calcium carbonate) or completely pigmented; others display stripes (parallel or perpendicular to the axis of growth), V-shapes, triangles, waves, spots, blotches, or some combinations of those. Some pigments may not be visible under ambient lighting but only, for example, under ultraviolet light (COX et al., 1969:71). Thus unpigmented or fully pigmented species may well have a more complex pattern than what we see. In fact, I submit that all species have some sort of complex patterns; in non- or completely pigmented species, more complex patterns should appear if appropriate detection methods were known and used.

Mathematical models of pigmentation patterns

The complex geometry of the pigmentation of many molluscan shells has attracted the attention of many theoreticians. Modelling of seashell patterns is facilitated by the fact that growth of the shell and formation of the pigmentation pattern occur only at the margin of the shell, which can be seen a1-dimensional structure. The final pattern on the shell can therefore be considered as a space-time diagram of the pigmentation process (There are a few exceptions, which need not concern us here; e.g. in species of the genus *Cypraea*, MEINHARDT, 1995:37-39). Pigmentation patterns can be explained by specific changes in the secreting activity of localised groups of cells along the mantle edge (COMFORT, 1951). If the pigment-secreting activity is stable in time, and discontinuous but stable in space, bands perpendicular to the shell margin are produced. If the activity is stable spatially but periodical, bands parallel to the shell margin are produced. If the activity spreads towards adjacent cells, triangles or inverted V shapes will be produced, depending whether the activity is inhibited or not after some delay. More complex patterns can be derived with slightly more complicated rules. Finding these rules and testing their capacity to generate the appropriate pattern by simulation has been rather successful.

WADDINGTON & COWE (1969) seem to have been the first to produce a model and a computer simulation for a pigmentation pattern, based on TURING's (1952) model for morphogenesis by autocatalysis and inhibition processes. Later, cellular automata models have been used (e.g. HERMAN & LIU, 1973; LINDSAY, 1982; WOLFRAM, 1983; GUNJI, 1990; KUSCH & MARKUS, 1996). The model developed by ERMENTROUT et al. (1986) is of special interest as it is based on some hypothetical neural activity in the mantle. Finally, the reaction-diffusion model of morphogenesis was expanded by MEINHARDT (1984, 1995). This model relies on the varying concentration of activating and inhibiting substances, which have typical production, decay and diffusion rates.

An interesting prediction of these models is that the formation of some pigmentation patterns is likely to be chaotic or undecidable. Turing reaction-diffusion models, which rely on variables taking real values, may be chaotic in the sense that a slight difference in the starting or boundary conditions may lead to a very different pattern. In the case of the cellular automata, which rely on variables taking only discrete values, the theory of computation applies. In some cellular automata (class IV of WOLFRAM, 1984) the slightest change in the starting condition will produce a completely different pattern. This property of discrete systems is termed undecidability, or computational irreducibility (WOLFRAM, 1984), meaning that the long term behaviour of such systems cannot be predicted from the initial conditions (this property is related to Turing's Halting Problem). KUSCH & MARKUS (1996) have found evidence for undecidability in several pigmentation patterns of actual seashells. Whether the processes generating the pigmentation patterns are chaotic or undecidable, the consequences that concern us here are the following. Firstly, the diversity of patterns within the population could be explained by small changes in starting conditions and/or rules or parameters. Secondly, in the case of bivalves, the frequent mirror-symmetry of complex pigmentation pattern between the two valves calls for an explanation. Without any sort of control mechanism, it is expected that the patterns on the two valves would diverge, due to small, unavoidable fluctuations. Third, the mollusc may have no control on the details of final pattern, only on its presence and general structure.

Where is the current pigmentation memorised?

The formation of the pigmentation patterns, as demonstrated by mathematical models, depends on adding to the current pattern according to specific rules. How does the mollusc 'know' what the current pattern is? Shell growth, hence pigmentation formation, may last for years and is frequently interrupted, from a few hours per day (e.g. tides) to several weeks or months (e.g. seasons). However, in many cases, the pigmentation pattern is not interrupted by those breaks: it is consistent across the growth marks. Thus when growth resumes, the pigmentation process must resume exactly where it was.

One possibility is that the memory is at the cellular level (LINDSAY, 1982; MEINHARDT, 1995). The pigment-producing cells on the mantle margins may retain their activity state between periods of growth. However, cells of the margin would have to be able to reposition themselves exactly as before; furthermore retaining their activity state may be difficult for periods sometimes lasting up to months. The reaction-diffusion system, as proposed by

TURING (1952) and applied to seashells (e.g. MEINHARDT, 1995) relies on precise variation in the concentration of activating and inhibiting substances. However, living molluscs encounter rather varying environmental conditions (e.g. temperature, water currents) under which it may be difficult to expect any stability, or regular changes in the concentration of the chemical substances as postulated by the model.

Another possibility is that the mantle does not memorise its state, but 'reads' the pigmentation pattern on the shell when needed, by contact chemoreception. Reading the pigmentation from the shell would have many advantages: the information is stable, readily accessible, and there is no energetic cost for maintenance. This 'reading' would be achieved by the rich neural and sensory network of the mantle (e.g. receptors described by SALEUDDIN 1979). ERMENTROUT et al. (1986:374) also proposed the ability of the mantle to taste the old pattern, to explain the alignment to the previous pattern, i.e. in some gastropods where new pigmentation stripes are initiated, on the next round of the shell, at the very position where a stripe appears on the previous round (see illustration in MEINHARDT, 1995:10). The ability to read the previous pattern may also be necessary to allow the stability of the pigmentation pattern despite the growth of the mantle: new cells must appear when the mantle grows (alongside the shell) and this organ is therefore continuously reorganised (cf. LINDSAY, 1982).

Regulation of the growth of the shell

Bivalves close their shells to escape from predators or from temporarily deteriorated environmental conditions. There is a selection pressure for perfect closure and, indeed, most bivalves appear completely sealed when the valves are closed. To achieve this, the two valves must be symmetrical in shape (at least along their margin) in order to close or interlock properly. We can expect some mechanism for developmental stability, otherwise random fluctuations of growth rate at the cellular level could be amplified and cause large (fluctuating) asymmetry in the size and shape of the two valves (cf. EMLEN et al., 1993), preventing proper closure. Environmental perturbations to the shell (erosion, fracture, predation attempts) may also induce an asymmetry unless some co-ordinated repair mechanism could restore the symmetry. Controlling and/or restoring the shape symmetry between the two valves requires a feedback (communication) mechanism between the two mantle lobes. I suggest again that the neural network of the mantle is the major component of this feedback activity. The nervous system is known to interact with the regulation of growth and regeneration of peripheral organs in molluscs (MOFFETT, 1991); it would also be involved in regulating the pigmentation activity (ERMENTROUT et al., 1986) and sensing the previous pigmentation pattern (see above). The existence of a feedback mechanism between the two valves would also explain the mirror-symmetry of their pigmentation patterns (MEINHARDT, 1995:10); without this feedback the two patterns would diverge rapidly during growth due to the chaotic dynamics of the pigmentation process. The presence of feedback between symmetrical structures to regulate their growth is also discussed by SWADDLE & WITTER (1997) in the case of primary feathers in birds and by EMLEN et al. (1993) on a theoretical basis. Interestingly, the latter authors suggest that feedback between sides should result in growth waves. I propose that this mechanism could explain the endogenecity of the microgrowth observed in seashells (BERARD et al., 1992; see above section on shell growth).

THE HYPOTHESIS

Pigmentation pattern is part of the developmental stability mechanism

My hypothesis is that the pigmentation pattern is intimately related to the growth process, more specifically to the mechanisms that control the growth of the shell in order to achieve the optimal shape (e.g. valve symmetry, in bivalves). I submit that pigmentation and growth are functionally related. Both co-occur temporally and spatially, as the pigmentation is deposited when and where the shell grows (see above). The same (richly innervated) organ, the mantle, would be responsible for growing the shell, depositing the pigment, and sensing the actual shell shape and the current pigmentation.

How could pigmentation pattern and developmental stability be related? The first possibility, as suggested by ERMENTROUT et al. (1986), is that the pigmentation pattern is a recording of the neural activity in the mantle, as an epiphenomenon. It is unclear why this neural activity should be recorded at all, but it is an interesting idea, consistent with my hypothesis that pigmentation and growth regulation are connected. The couplings and feedbacks needed to regulate the growth of the shell may well constitute a system with a non-linear dynamic, the kind of dynamic from which we expect complex and diverse patterns, such as those found in the pigmentation of seashells.

I suggest that the pigmentation pattern is not an epiphenomenon but is used by the mantle as marks to locate position on the shell margin. The need for position marks comes from the fact that the shell margin would otherwise be a uniform one-dimensional structure, along which, however, growth rate must be unequal and adjusted in order to achieve the desired shape. In order to regulate the growth of the shell, the mantle needs to modulate the deposition of new shell material at precise positions along the shell margin. Pigmentation of the shell would provide stable marks that could be read by the mantle at anytime, even after interruption of growth by the endogenous daily rhythm and/or environmentally driven cycles (tides, seasons). In other words, pigmentation would create heterogeneity on the shell that the mantle could use to position

its activity. This primary function would easily be compatible with secondary functions such as crypsis or thermoregulation; it is also compatible with a high degree of intra- and interspecific variability in the pigmentation patterns, and is compatible with the fact that pigments are taken from and vary with the diet.

The role of pigmentation patterns in development would explain their ubiquity. Most species have complex pigmentation patterns, and if not, I suggest that other substances are used for marking the shell surface, which may not be visible to the naked eye. Those substances need not be pigments, but need to be able to mark the shell material in a durable way and to be easily sensed ('tasted') by the mantle. Those pigments or other substances may be waste products and/or taken from the diet. The large variety of pigmentation pattern, both within and between species would be explained by the fact that there may be little selection on the structure of the pattern, as long as it allows positioning. However, some patterns may be more efficient than others for facilitating the mantle to position itself. There might be selection for the kind of regular or complex patterns often seen, but this remains an area of investigation.

The hypothesis presented here remains speculative and needs to be tested. Fortunately some bivalves species can be bred and/or grown in the laboratory (or in aquaculture set-ups), allowing for experimentation. My hypothesis could be tested by perturbing the shell pigmentation and/or the mantle. If the existing pigmentation pattern is altered, perhaps by transplanting part of the shell or directly altering the pigmentation pattern with chemicals, then we expect to observe change in the growth and ultimately the shape of the shell, leading to abnormal asymmetry in shape. Experiments on the mantle could involve the suppression of the communication between the two mantle lobes (by using inhibitors, by sectioning some neural connection – see MOFFETT 1991). Here we would expect abnormal changes in both the pigmentation pattern (asymmetry in species having mirror-symmetry) and the shell shape. Those bivalve species that have mirror-symmetry in their pigmentation pattern may be used to find the actual mechanism of intervalve communication, using a range of neurotoxins or inhibitors and checking whether the symmetry in pigmentation is still achieved or not. In this way, pigmentation patterns may provide a useful handle to study the developmental mechanism of shell formation. Finally, another prediction of the hypothesis that could be verified is the presence of 'hidden' patterns in those species where no pattern is visible under ambient light. Patterns should exist in every species and it would only be a matter of finding a suitable technique to observe them.

In conclusion, I propose to have a new look at the pigmentation patterns of seashells (and its mathematical modelling) on the basis of two ideas. The first concerns the mechanism: the mantle, through its neural and sensory network, regulates the deposition of new pigmentation by reading the previous pigmentation on the shell. The second concerns the function: the pigmentation pattern is related to the developmental regulation of shell growth. My hypothesis is speculative and there are a number of open questions. However, experimental tests can be designed and should provide a better understanding of the evolutionary puzzle of seashell pigmentation patterns. Although centred on (marine) bivalves, my hypothesis may well be valid for other molluscs, marine, freshwater or terrestrial, although it is clear that secondary functions such as crypsis and warning signals have evolved in some species. In those cases, either the pattern for crypsis or warning could be used for growth regulation as well, or non-pigmented markers could be used.

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Characteristics of different populations of the gudgeon (*Gobio gobio* L.) in Flanders, Belgium

Alain Dillen [1], Lieven Bervoets [1], Gudrun De Boeck [1], Marcel Eens [2] and Ronny Blust [1]

[1] **Department of Biology, University of Antwerp (R.U.C.A.), Groenenborgerlaan 171, B-2020 Antwerp, Belgium**
[2] **Department of Biology, University of Antwerp (U.I.A.), Universiteitsplein 1, B-2610 Wilrijk, Belgium**

ABSTRACT. We examined the possible effects of isolation on population characteristics of the gudgeon, *Gobio gobio* L., 1758. Isolated populations appeared to have significantly lower densities than non-isolated populations. Length-frequency distributions showed that most non-isolated populations had a healthy composition, with a larger number of young animals, and a smaller number of older animals. However, for some isolated populations this was not the case, despite the fact that some of them had high densities. Four condition factors were compared between isolated and non-isolated populations. They showed no relationship with isolation, although significant differences among populations were found. Fluctuating asymmetry (FA) was determined using metric and meristic traits. The meristic traits could not detect the presence of FA, but the metric traits showed that FA was present in some populations. Significant differences were found among populations, but the FA could not be related to isolation. The growth rate showed significant differences among populations, but was not related with isolation. The maximum swimming speed, oxygen consumption, ammonia production, and ammonium quotient were assessed in the laboratory. None of these parameters showed a relationship with isolation, but there were significant differences among populations. Overall, our results show that population density and distribution seem to be affected by isolation, while for the other parameters no effects of isolation were found. However, significant differences among populations were found for all other parameters.

KEY WORDS: gudgeon, isolation, condition, population structure, fluctuating asymmetry, swimming capacity, oxygen consumption, ammonia excretion.

INTRODUCTION

Destruction and fragmentation of natural habitats by man is the major reason for the worldwide decrease in biodiversity (QUINN & HARRISON, 1988). While the adverse effects of anthropogenic habitat fragmentation are well documented for most native terrestrial plants and animals, its importance as an isolating mechanism in stream-dwelling species has been largely overlooked (DODD, 1990). However, the natural area of most Flemish fish species, such as the gudgeon (*Gobio gobio* L., 1758), is often highly fragmented by the presence of barriers (e.g. dams, weirs, water mills, pumping-engines, pollution) (UTZINGER et al., 1998), leading to the isolation of populations. In many species, the population size will decrease due to isolation, making these populations more vulnerable to extinction (SOULÉ, 1983; STEFANN-DEWENTER & TSCHARNTKE, 1999). A possible explanation for this decrease could be that some species are no longer able to find all of their resources (food, spawning area, shelter,..) in the reduced habitat (SPELLERBERG, 1996). Another consequence of isolation is that the exchange of individuals and genetic material between populations might decrease (LANDE, 1988; LANDE & BARROWCLOUGH, 1990). Especially in small populations, this will lead to the depletion of genetic variation (i.e. genetic erosion). In isolated populations, inbreeding will increase as population size decreases, resulting in a reduced fitness (inbreeding depression) and in heterozygosity loss (ALLENDORF, 1983; LANDE & BARROWCLOUGH, 1990). Several studies have demonstrated a reduced body weight and volume, a lower fitness and a lower fecundity in isolated populations compared to non-isolated populations (LANDE & BARROWCLOUGH, 1990; WAUTERS et al., 1996; WIGGINS et al., 1998; TANAKA, 2000). The lower body weight and volume could have a negative influence on the physiological condition of the animals (WAUTERS et al., 1996). For

Corresponding author: A. Dillen, e-mail: adillen@ruca.ua.ac.be

instance, fishes with a lower body weight might have less energy reserves and/or a lower swimming capacity. Condition factors, derived from the length-weight relationship, are often used as bio-indicators for environmental stress, and are thought to be related to fitness (LAMBERT & DUTIL, 1997; DUTIL et al., 1998; SUNEETHA et al., 1999). A lower condition might influence the tolerance of fishes to an additional stress, such as lower oxygen concentrations. Furthermore, a reduced heterozygosity might result in a higher fluctuating asymmetry (FA) (i.e. the random deviations from perfect symmetry in bilaterally paired traits) (LEARY & ALLENDORF, 1989; PALMER, 1994).

We examined the possible effects of isolation on the population characteristics of the gudgeon, by: (1) comparing condition factors and growth rates between isolated and non-isolated populations, (2) studying the effects of isolation on the population structure, and (3) studying the effect of isolation on physiological parameters that could be related to condition (e.g. swimming capacity, oxygen consumption and ammonia production).

MATERIAL AND METHODS

Sampling

We sampled sites with a comparable water quality, throughout Flanders (Fig. 1), using electrofishing. Sites were considered to be isolated if there were any obstructions (higher than 15 cm) or a strong pollution present downstream from the site. Information about water quality was obtained from the online database of the Dutch Environmental Company (http://www.vmm.be). The sites were sampled from 22/10/99 to 28/02/00 to investigate the population structure. From 25/02/99 to 16/03/00 four isolated and five non-isolated sites (both with high gudgeon densities) were sampled to obtain fish for the laboratory study.

Test species

The gudgeon (*Gobio gobio*) belongs to the family of the cyprinids and is widespread in European rivers. It was selected as test species for several reasons. First of all gudgeons are abundant and widespread in the Flemish water courses (VANDELANNOOTE et al., 1998), implying that sampling would not endanger its survival. Secondly, since gudgeons have a limited home range, individuals caught at a specific site can be considered to be representative for the selected sampling site (STOTT, 1967; STOTT et al., 1963). Thirdly, gudgeons have not been introduced in the selected study area.

Field study

During each sampling session, we used depletion fishing to estimate population size. At each site, three subsequent captures were performed, and the fish caught during each capture were kept separately in plastic tanks. Population size was then calculated according to two methods: the method of DeLury, as described by LAURENT & LAMARQUE (1975), and the method of Zippin, as described by SOUTHWOOD (1968). The fork length of the fish was measured to the nearest mm, and the weight was measured to a precision of 0.1 g, using an electronic balance (KERN 442-43). Based on the length-weight relationship, four condition factors were calculated (WEATHERLY, 1972; BAGENAL, 1978). This relationship followed the equation: $W = a * FL^b$ [1] (with W = weight and FL = fork length).

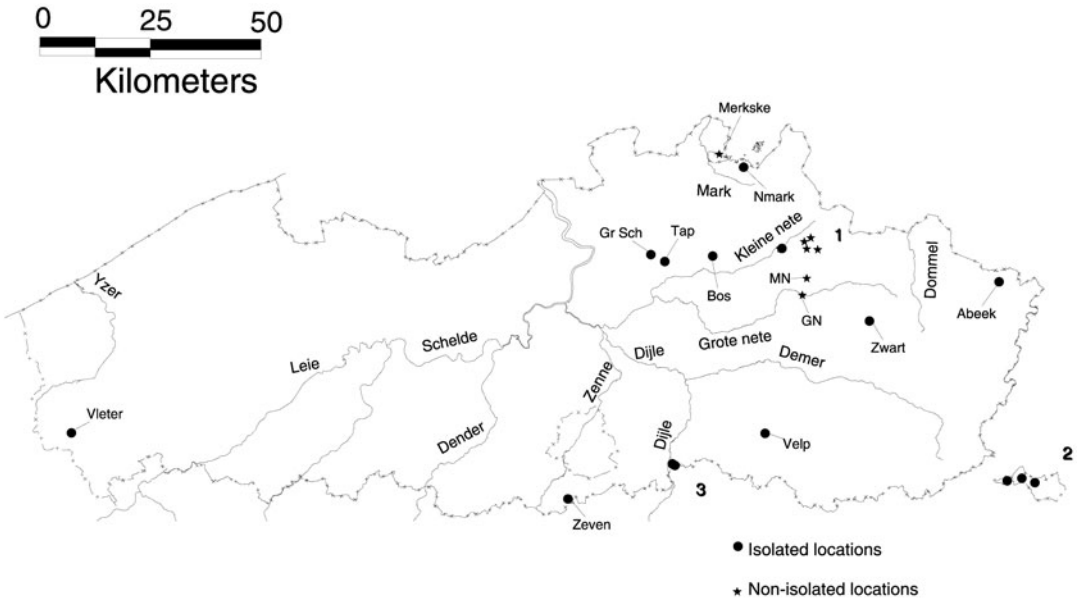

Fig. 1. – Sampling sites for gudgeon in Flanders. Used abbreviations: Nmark = Noordermark, MN = Molse Nete, Gr Sch = Groot Schijn, Tap = Tappelbeek, GN = Grote Nete, Vleter = Vleterbeek, Zwart = Zwarte Beek, Zeven = Zevenborrebeek.

Fulton's condition factor (FULTON) was calculated by the formula: $K = (100*W)/FL^3$. Two adapted condition factors were calculated by $K' = (100*W)/FL^b$ (b as the value calculated in equation [1]). The first adapted condition factor (ADCF 1) was calculated using the b-value for each population separately, while the second one (ADCF 2) was calculated using the overall b-value. The relative condition factor from Le Cren (LECREN) was calculated by: $K'' = W/W^{exp}$; W^{exp} being the weight of the fish as expected based on equation [1].

From each gudgeon, six scales were taken from above the lateral line (left side). These scales were dried for at least three days, then washed in a 0.1 N NaOH solution, and finally they were mounted on a microscopic slide. The annual rings on the scales were counted using a microprojector (Heerbrug Projectina type 4002). This allowed us to determine the age, from which the growth was subsequently calculated using the 'back calculation' method of Fraser-Lee (BAGENAL, 1978). The relationship between fork length (FL) and scale radius (SR) follows the equation: $FL = a+b*SR$ [2]. From this relationship, the fork length at a given age n (FL_n) can be calculated by the formula:

$$FL_n = \frac{AR_n}{SR} * (FL - a) + a$$

in which AR_n is the distance between the centre of the scale and the n-th annual ring, and a is the intercept from equation [2].

Fluctuating asymmetry (FA) was studied by examining both metric and meristic bilateral traits. The meristic traits were counted in the field: (SCAL); the number of scales above the lateral line, and (PELVRAY); the number of rays counted at the base of the pelvic fin. The metric traits were the height (H) and the width (W) of the fourth scale along the lateral line, counted from head to tail. For these traits, the left and right side were measured repeatedly (sequence left-right-left-right) to the nearest 0.1 mm, using a microprojector (Heerbrug projectina type 4002). Repeated measurements were necessary to distinguish true FA from measurement error (LENS et al., 1999).

Small fin clips were taken from all gudgeons for further DNA-analysis (during this study, the primers for gudgeon DNA were not yet available).

Laboratory study

Four isolated and five non-isolated locations were studied. From each location, seven gudgeons, ranging in size from 6-13 cm, were taken to the lab and acclimated for three days at a temperature of 15° C, and at a photoperiod of 10 h light/ 14 h darkness.

The first two days of the acclimation period, fish were fed with midge larvae.

All experiments were carried out in seven respirometers (Fig. 2), analogous with the Blazka-respirometer (BEAMISH et al., 1989).

To determine the critical swimming speed, seven fish were measured and weighed as described before, and then placed in the respirometers. The respirometers were connected to a continuous flow system to ensure sufficient oxygen concentrations. The front side of each respirometer was covered with a black plastic, in order to minimize the disturbance caused by the observer. After these preparations, the fish were left alone for an hour to allow them to calm down. Then a water current of 10 cm/s was applied. Every 20 minutes, the velocity was raised by 5 cm/s (KEEN & FARRELL, 1994; LAUFF & WOOD, 1996; ALSOP & WOOD, 1997). When a fish became exhausted, it was pushed against the back membrane. When this happened, the current was lowered for a few seconds and then reinstalled to its original velocity. If the fish was pushed against the membrane a second time, the experiment was stopped and the time and velocity of the occurrence were noted. The critical swimming speed (U_{crit}) was then calculated as follows (KEEN & FARRELL, 1994; LAUFF & WOOD, 1996; ALSOP & WOOD, 1997; MCDONALD et al., 1998):

$$U_{crit} = U_p + \left(\frac{T}{t}\right) + dU$$

With U_p as the last velocity at which the fish was able to swim during a complete time interval, T = the time the fish swam during the velocity of exhaustion, and t = time interval (20 minutes), dU = speed increment (5 cm/s). The critical swimming speed was then expressed in units of body length/s.

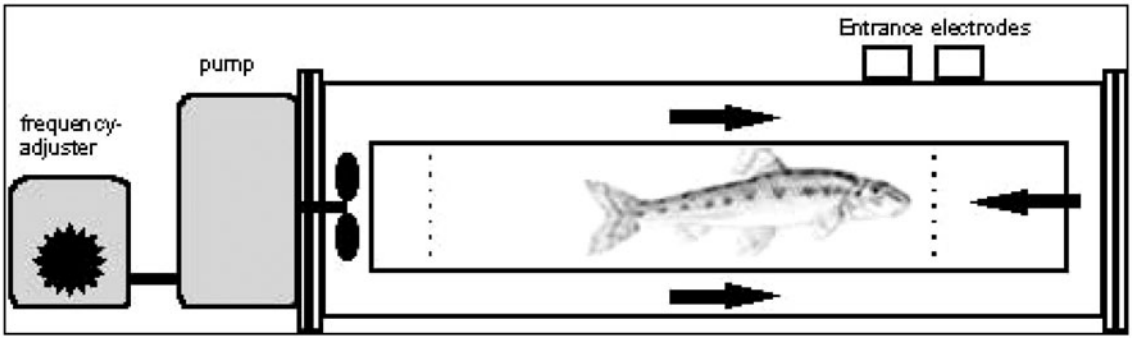

Fig. 2. – Schematic figure of the respirometer. The black arrows indicate water flow, the dotted lines represent membranes, which keep the fish in the inner tube and allow water to flow through the inner tube.

The day after determination of the critical swimming speed, oxygen consumption and ammonia excretion were measured. For this, the fish used in the previous experiment were held in the respirometers over night, with the respirometers connected to a continuous flow system. For each population, one fish was removed, so that there was one respirometer for the blanco measurements. The fish were forced to swim at a velocity equal to $^1/_4$ of U_{crit} for two hours. During this experiment, the respirometers were closed so that no atmospheric oxygen could enter the system. Oxygen concentrations were continuously measured using WTW CellOx 325 electrodes, which were connected to a computer for automatic reporting (WINDMILL 4.06 software). At the beginning and the end of each experiment, 4 ml water samples were taken in triplicate to measure total ammonia concentration. This measurement was done using the hypochlorite-phenol method (GRASSHOFF, 1976). After the experiments, FA and growth were determined for the tested fishes as described above.

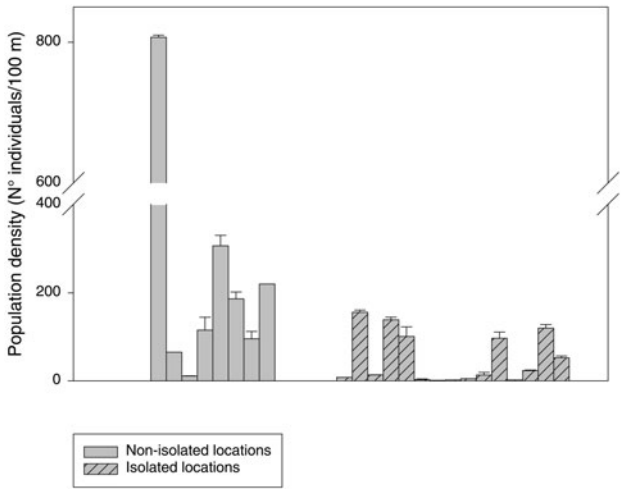

Fig. 3. – Population densities for the gudgeon. Dashed bars represent isolated populations. Densities are expressed as number of individuals/ 100 m.

Statistical analysis

Normal distribution of data was tested using the Kolmogorov-Smirnov test, and homogeneity of variance was tested using the Bartlett test (ZARR, 1996; DYTHAM, 1999). If both assumptions were fulfilled, one-way ANOVA was performed to check for differences among locations, followed by a Tukey-Kramer test. If one of these assumptions was not fulfilled, a non-parametric test (Kruskall-Wallis test for differences among populations and Mann-Whitney-U test for differences between isolated and non-isolated populations), followed by a post hoc Dunn's test, was used. Correlations were tested with Spearman-rank correlation. All these tests were performed in Graphpad Instat® 3.01. The analysis of FA was performed using the mixed procedure in SAS, in which individuals were defined as repeated statement. Restricted maximum likelihood (REML) test was used to distinguish true FA from measurement error (LENS & VAN DONGEN, 1999). An F-test was used to distinguish FA from directional asymmetry.

RESULTS

Field study

Using the method of Delury and the method of Zippin, very similar results were obtained. Population densities (Fig. 3) were significantly higher in non-isolated than in isolated populations (Mann-Whitney U, P = 0.0098). This was still the case if the site 'Witte Nete', with an exceptionally high population density (807 ind./100 m), was not included in the analysis (P = 0.022). This clearly shows that the population density is affected by isolation. While nine of the fifteen isolated locations had population densities lower than 30 individuals per 100 m, there was only one of the eight non-isolated locations with such a low density.

Length-frequency distributions are given in Figs 4a-4b. For three isolated locations the length-frequency distribution is not shown, because at each location only few gudgeons were caught (Nethen 1: 1 gudgeon, fork length 106 mm; Nethen 2: 1 gudgeon, fork length 139 mm and Voer: 2 gudgeons, fork lengths 122 and 134 mm). Obviously, populations with small individual numbers have a very narrow distribution of length classes, consisting mainly of larger and older fish. These populations are all isolated populations. Furthermore, only two isolated locations with a population density larger than 30 individuals per 100 m show a population structure that consists of a relatively large number of young (small) individuals, and a smaller number of larger (older) individuals (i.e. Abeek and Noordermark). The other isolated high density populations have a larger number of older individuals compared to young individuals (i.e. Vleterbeek, Berwijn, Groot Schijn and Velp). For the non-isolated populations, a population distribution with a relatively large number of young animals was found in all cases, even for the one location with a low density (i.e. Desselse Nete point 1).

The meristic traits could not be used to detect FA; for both traits (SCAL and PELVRAY), deviations from the normal bilateral symmetry were only found in two fishes. However, the metric traits (H and W) allowed us to detect FA. The real asymmetry was always significantly larger than measurement error (REML test, all P-values < 0.001). The average values of the assigned asymmetry were never significantly different from zero (F-test, all P > 0.1), meaning that the asymmetry was due to FA and not to directional asymmetry. For both traits, there were significant differences (KW, P < 0.0001) in FA among populations (Figs 5a-5b), but these differences could not be related to isolation due to insufficient data for the non-isolated populations (due to lack of time, we were only able to obtain data for the site 'Witte Nete').

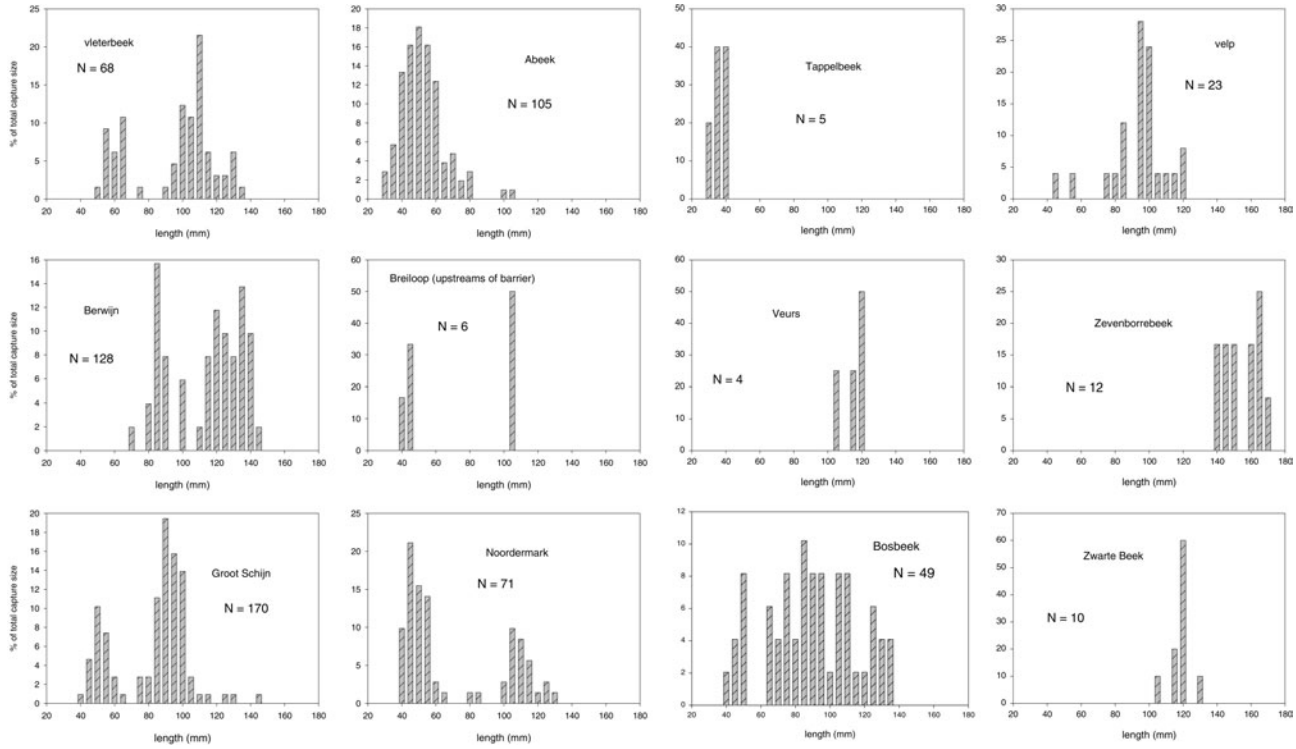

Fig. 4a. – Length-frequency distributions for isolated populations of the gudgeon. N is the total number of fish caught at the site during the sampling; length intervals are taken at 5 mm.

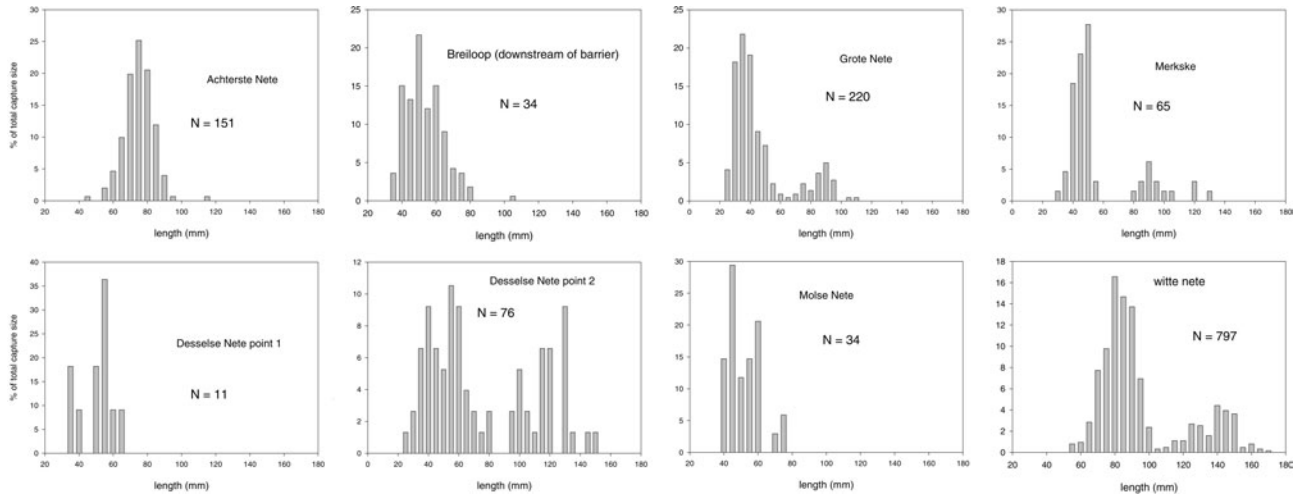

Fig. 4b. – Length-frequency distributions for non-isolated populations of the gudgeon. N is the total number of fish caught at the site during the sampling; length intervals are taken at 5 mm.

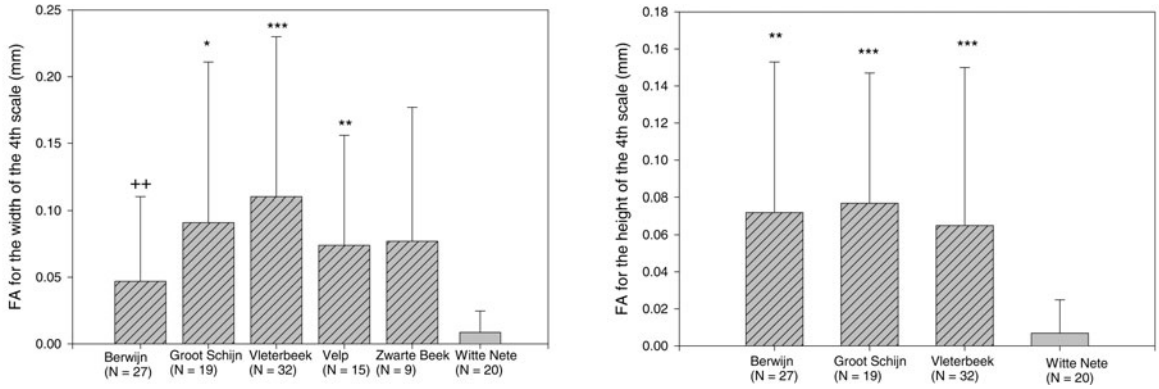

Fig. 5. – *a*. (left). Means and standard deviations of the fluctuating asymmetry for trait W. *b*. (right). Means and standard deviations of the fluctuating asymmetry for trait H. Dashed bars represent isolated populations. ++: significantly lower FA than Vleterbeek ($P < 0.01$); *: significantly higher FA than Witte Nete (*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$).

The back-calculated fork lengths showed a linear relationship with age, allowing us to determine growth rates by linear regression. The growth rates (Fig. 6) differed significantly among populations (ANCOVA, ddfm = 4 and 1162, $F = 36.37$, $P < 0.001$), but could not be related to isolation (Table 1).

For the calculation of the condition factor of Fulton (FULTON), the data were divided into five length classes (<60 mm, 60-80 mm, 80-100 mm, 100-120 mm and > 120 mm). For all length classes, the results were similar. A Kruskall-Wallis ANOVA showed that there were significant differences among populations (for each length class : $P < 0.01$), and a Dunn's post hoc test showed that the FULTON-value was significantly higher for one isolated population (i.e. Vleterbeek) (Table 2), but the condition factor of Fulton could not be related to isolation.

There were also significant differences among populations for the adapted condition factors (i.e. ADCF 1 and 2) (for both: $P < 0.001$), but neither one of these condition factors could be related to isolation. For both of the adapted condition factors, the Vleterbeek population had the highest condition, but there were also significant differences among the other populations (Table 3).

Similar results were found for the condition factor of Lecren (LECREN): there were significant differences among populations ($P < 0.001$), but they could not be related to isolation. The LECREN value was also the highest for the Vleterbeek population.

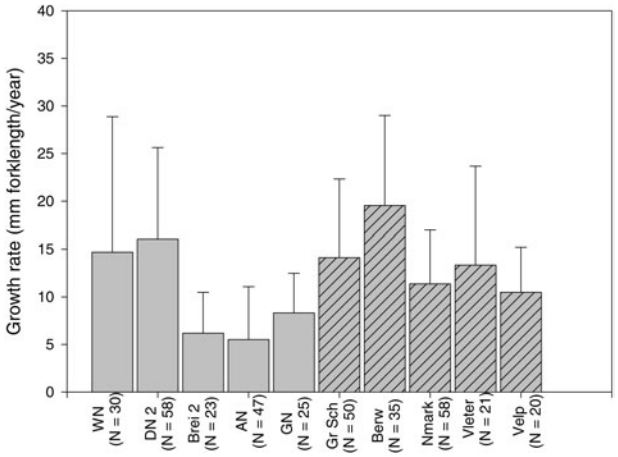

Fig. 6. – Means and standard deviations of the growth rates (in mm fork length/year) for gudgeon populations. Dashed bars represent isolated populations. N = number of fishes used to determine age and growth.

TABLE 1

Differences in growth rate among populations. ns: not significant; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$. [i]: isolated populations.

	WN	**DN 2**	**Brei 2**	**AN**	**GN**	**Gr Sch[i]**	**Berwijn[i]**	**Nmark[i]**	**Vleter[i]**	**Velp[i]**
WN	---									
DN 2	ns	---								
Brei 2	***	***	---							
AN	***	***	ns	---						
GN	***	ns	ns	ns	---					
Gr Sch[i]	ns	ns	***	***	***	---				
Berwijn[i]	***	*	***	***	***	***	---			
Nmark[i]	ns	***	***	***	ns	ns	***	---		
Vleter[i]	ns	ns	***	***	**	ns	***	ns	---	
Velp[i]	*	***	*	***	ns	*	***	ns	ns	---

TABLE 2

Results of the Dunn's multiple comparisons test for FULTON, for the length class < 60 mm. The results for the other length classes are similar. [i]: isolated populations. ns: not significant; *: P < 0.05; **: P < 0.01; ***: P < 0.001.

	Desselse Nete	**Merkske**	**Molse Nete**	**Achterste Nete**	**Breiloop 2**	**Grote Nete**	**Noordermark[i]**	**Groot Schijn[i]**	**Abeek[i]**	**Vleterbeek[i]**
Desselse Nete	---									
Merkske	ns	---								
Molse Nete	ns	ns	---							
Achterste Nete	ns	ns	ns	---						
Breiloop 2	ns	ns	ns	ns	---					
Grote Nete	ns	ns	ns	ns	ns	---				
Noordermark[i]	ns	ns	ns	ns	ns	ns	---			
Groot Schijn[i]	ns	ns	ns	ns	ns	ns	ns	---		
Abeek[i]	*	ns	ns	ns	ns	ns	ns	ns	---	
Vleterbeek[i]	***	***	**	*	***	***	***	**	**	---

TABLE 3

Results of the Dunn's multiple comparisons test for ADCF 1. The results for the ADCF 2 are similar. [i]: isolated populations. *: P < 0.05; **: P< 0.01; ***: P < 0.001.

	Witte Nete	**Desselse Nete**	**Velp[I]**	**Abeek[i]**	**Groot Schijn[i]**	**Achterste Nete**	**Berwijn[i]**	**Grote Nete**	**Breiloop 2**	**Molse Nete**	**Merkske**	**Noordermark[i]**	**Vleter[i]**
Witte Nete	---												
Desselse Nete	***	---											
Velp[i]	ns	***	---										
Abeek[i]	***	***	***	---									
Groot Schijn[i]	***	ns	***	**	---								
Achterste Nete	ns	***	ns	***	***	---							
Berwijn[i]	***	***	***	ns	*	***	---						
Grote Nete	***	***	***	ns	**	***	ns	---					
Breiloop 2	***	ns	***	***	ns	***	***	***	---				
Molse Nete	ns	***	***	***	***	ns	***	***	***	---			
Merkske	***	ns	***	***	ns	***	***	***	ns	***	---		
Noordermark[i]	ns	*	***	***	***	***	***	***	*	***	ns	---	
Vleter[i]	***	***	***	***	***	***	***	***	***	ns	***	***	---

Laboratory study

The results for the critical swimming speed are shown in Table 4. Significant differences were found among populations (P < 0.01), and the Dunn's multiple comparisons test showed that the populations from Velp (isolated) (P < 0.01) and from Witte Nete (non-isolated) (P < 0.05) had lower mean critical swimming speeds than the population from Groot Schijn (isolated). Therefore, the critical swimming speed did not seem to be related to isolation.

The oxygen consumption (Table 5) also was not related to isolation, although significant differences were found among populations (P = 0.007). The populations from Merkske (non-isolated) (P = 0.006), Groot Schijn (isolated) (P = 0.006) and Achterste Nete (non-isolated) (P = 0.004) had higher oxygen consumptions than the population from Velp (isolated). Also, the populations from Merkske (isolated) (P = 0.02), Groot Schijn (isolated) (P = 0.02) and Achterste Nete (not-isolated) (P = 0.03) had higher oxygen consumptions than the Abeek population (isolated).

There were significant differences among populations for the ammonia production (P = 0.0001), but the ammonia production (Table 6) was also not related to isolation.

TABLE 4

Means and standard deviations of the critical swimming speed (U_{cr}), in body lengths/s. [i]: isolated populations. For each population, N = 7.

Population	Critical swimming speed	
	Mean	Stdev
Velp[i]	2.99	1.15
Abeek[i]	5.48	1.54
Groot Schijn[i]	6.98	0.65
Bosbeek[i]	6.19	2.03
Witte Nete	3.62	1.22
Merkske	5.66	0.89
Achterste Nete	6.72	2.03
Desselse Nete 1	4.58	1.86
Desselse Nete 2	4.09	2.06

TABLE 5

Means and standard deviations of the oxygen consumption, in umole*g^{-1}*h^{-1}. [i]: isolated populations. For each population, N = 6.

Population	Oxygen consumption	
	Mean	Stdev
Velp[i]	6.29	1.13
Abeek[i]	4.95	2.55
Groot Schijn[i]	13.60	7.68
Bosbeek[i]	11.65	4.60
Witte Nete	9.65	7.00
Merkske	13.60	7.68
Achterste Nete	12.25	6.28
Desselse Nete 1	7.64	3.12
Desselse Nete 2	7.38	1.78

TABLE 6

Means and standard deviations of the ammonia excretion, in umole*g^{-1}*h^{-1}. [i]: isolated populations. For each population, N = 6.

Population	Oxygen consumption	
	Mean	Stdev
Velp[i]	0.27	0.09
Abeek[i]	0.21	0.12
Groot Schijn[i]	0.50	0.19
Bosbeek[i]	0.34	0.10
Witte Nete	0.45	0.19
Merkske	0.36	0.10
Achterste Nete	0.55	0.14
Desselse Nete 1	0.26	0.07
Desselse Nete 2	0.24	0.13

Correlations

None of the condition factors was correlated with the physiological parameters from the laboratory study, nor with the FA (all P > 0.05). The FA, the oxygen consumption and the critical swimming speed were not related with any of the other parameters (all P > 0.05).

DISCUSSION

Field study

Population densities were higher in non-isolated than in isolated populations. Perhaps, this could partially be explained by the width of the selected sites: most of the isolated sites were smaller than the non-isolated sites. However, the range of widths was rather small (2-8 m), and in broader rivers, electrofishing becomes less efficient, potentially leading to an underestimation of the population size. Another explanation for the difference in population size could be given by adverse effects of isolation. Inbreeding depression, demographic stochasticity and environmental stochasticity are possible causes for a decline in population size (LANDE, 1988; WIGGINS et al., 1998).

For the estimation of the population densities, two different methods were used. The results from both methods were very similar, but the method of Zippin allowed us to calculate the standard error as well, while the method of DeLury did not. Although LAURENT & LAMARQUE (1975) claim that the DeLury method may be used to calculate standard errors, it is not statistically correct, as the X and Y values used to make the estimate are not independent of each other (SOUTHWOOD, 1966). Standard errors are required to perform statistical analysis, so it is preferable to use the method of Zippin.

A healthy population structure consists of a relative large number of small fish and a small number of larger fish (NIKOLSKII, 1980; SCHLOSSER, 1982; MATTHEWS, 1998). While for all non-isolated populations a healthy population structure was found, this was only the case in two isolated populations. This suggests that isolation affects the population structure and density. Maybe individuals from the non-isolated populations have a higher reproductive success, or they suffer less from genetic erosion than the isolated populations. Future DNA-analyses of fin clips will allow us to examine whether there are genetic differences between isolated and non-isolated populations.

The meristic traits could not detect the presence of FA in this study. The use of meristic traits in FA-analysis has the disadvantage that they result in integer values. This means that FA will not be detected using only meristic traits, unless the amount of FA is very large, which only occurs in very extreme conditions (AMES et al., 1979; PALMER, 1994). However, the metric traits showed that FA was present in some populations. Significant differences

were found among populations, but the FA could not be related to isolation due to insufficient data for the non-isolated populations. Nevertheless, the fact that we were able to detect the presence of FA in some populations, might indicate that FA is a useful tool for studying effects of isolation.

No relationship was found between isolation and growth rate. The highest growth rate was found for an isolated water course, the Berwijn, which might be explained by the high productivity (food availability) of this river (Bervoets, unpublished data). Possible effects of isolation on the growth rate might be shaded by effects of other environmental influences, such as food availability, temperature, flow regime, etc… The effects of sex on growth were not investigated in this study, but LOBON-CERVIA et al. (1991) demonstrated that gender has little or no influence on the growth rate of the gudgeon.

Condition factors have been linked to survival of fish (BOOTH & HIXON, 1999). For all condition factors, significant differences were found among populations. This might indicate ecological differences among the populations, since condition factors are thought to be related to the fitness of fish. However, none of the condition factors was related to isolation. The condition factor of Fulton reached lower values in this study (values ranging from 0.8 to 1.4) than in the study of LOBON-CERVIA et al. (1991) (values ranging from 1.0 to 1.6). This might be explained by the warmer climate in Spain.

Laboratory study

The critical swimming speed showed no relation with isolation. However, we should emphasize that significant differences among populations were present. A possible complementary parameter for the swimming speed is the swimming activity (i.e. the time the fish actually swim during a given period). Similar results were found for the oxygen consumption and ammonia excretion. The swimming performance is related to survival (escaping from predators, searching for food) and to reproductive success (reaching suitable spawning areas, finding a mate) (KEEN & FARRELL, 1994; LAUFF & WOOD, 1996; ALSOP & WOOD, 1997; MCDONALD et al., 1998). Therefore, differences in critical swimming speed may have implications for the fitness of fish. Oxygen consumption and ammonia excretion are often used as endpoints in toxicity tests, and have proven to be very sensitive measures for stress (KUTTY, 1972; DE BOECK et al., 1995). The oxygen consumption values found in this study lie within the same range found for other cyprinids (5-15 $\mu mole.g^{-1}.hour^{-1}$) (FORSTNER & WIESER, 1989). DE BOECK et al. (1995) found ammonium excretions for carp up to 0.7 $\mu mole.g^{-1}.hour^{-1}$, which is also comparable with those found for gudgeon in this study.

All three physiological parameters have ecological relevance. The fact that for the three physiological parameters, significant differences were found among populations, might be an indication that there are ecological differences among these populations. It is possible that food availability has an important influence on these parameters, but unfortunately we did not measure food abundance.

Conclusions

Population density and structure were the only population characteristics that were affected by isolation. This could make isolated populations even more vulnerable to extinction through demographic and/or genetic and/or environmental processes. For the other population characteristics, we could not detect clear differences between isolated and non-isolated populations. However, there were significant differences among populations for all other parameters.

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The male dimorphism in the dwarf spider *Oedothorax gibbosus* (Blackwall, 1841) (Erigoninae, Linyphiidae, Araneae): Results of laboratory rearing experiments

Danny Vanacker[1], Jean-Pierre Maelfait[1,2] and Léon Baert[3]

[1]**University of Ghent, Laboratory of Animal Ecology, Zoogeography and Nature Conservation, Ledeganckstraat 35, B-9000 Ghent**
[2]**Institute of Nature Conservation, Kliniekstraat 25, B-1070 Brussels**
[3]**Royal Institute of Natural Sciences, Vautierstraat 29, B-1000 Brussels**

ABSTRACT. *Oedothorax gibbosus* is a dwarf spider bound to oligo- and mesotrophic alder carrs. Two male morphs occur: *gibbosus*, characterised by a hunch and a hairy groove on its carapace, and *tuberosus* without these features. The hairy groove is supposedly of importance during gustatorial courtship behaviour.

Earlier studies indicated that this dimorphism was presumably determined by a di-allelic gene only expressed in the male sex. Allele G for *gibbosus* is dominant over allele g for *tuberosus*. An elaborate laboratory rearing experiment was set up to test this hypothesis, which was based on only a small number of observations. Analysis of a large number of family trees was in agreement with this model of inheritance.

A small fraction of the reared spiders (5%) needed a fifth moult to reach the adult instar. This is the first time that an exception to the normal number of four moults in dwarf spiders of the subfamily Erigoninae has been observed. The dwarf spiders that moulted five times also hatched significantly earlier, and this probably made an additional moult necessary.

The juvenile phase of the *gibbosus* males was significantly longer than that of the *tuberosus* males. The juvenile phase is the period between the emergence of the spiders and the last moult.

Two possible mechanisms compensating for the advantage of *gibbosus* in sexual selection were observed: sex ratio distortion in favour of the female sex in *tuberosus*-genotypes, and shorter juvenile development.

KEY WORDS: Araneae, Erigoninae, male dimorphism, sexual selection, one-locus system, fifth moult, juvenile development, sex ratio distortion, Q10 rule.

INTRODUCTION

Oedothorax gibbosus is a rare dwarf spider species in Flanders, which occurs in wet to very wet habitats (De Keer & Maelfait, 1989; Alderweireldt, 1992) such as oligo- and mesotrophic alder carrs. They occur between litter and mosses in the immediate vicinity of open water. The males are smaller than the females. The abdomen of *Oedothorax gibbosus* is black and gleaming, and while walking the dwarf spider sometimes raises the abdomen. When the dwarf spider reaches adulthood it is approximately 3 mm in length.

Corresponding author: D. Vanacker, e-mail: danny.vanacker@rug.ac.be

Oedothorax gibbosus is characterised by male dimorphism. The *gibbosus* morph has a protuberance on the last third of the carapace, anterior to which is a deep notch surrounded and filled by long black silky hairs. This hairy groove probably secretes a fluid that is important for the gustatoric courtship behaviour, and *gibbosus* would therefore have a reproductive advantage (Heinemann & Uhl, 2000). The *tuberosus* morph does not have these features and its carapace is more or less convex. Previously, both morphs were considered to be two different species, *Oedothorax tuberosus* (Blackwall, 1841) and *Oedothorax gibbosus* (Blackwall, 1841), which could only be distinguished on the basis of the morphology of the males. De Keer & Maelfait (1988) proved the male dimor-

phism in *Oedothorax gibbosus*: both morphs hatched from one cocoon collected in the field.

Polymorphism according to FORD (1945) is the coexistence of two or more discontinuous genetically-determined morphs. Dimorphism within one sex is rare, and is generally related to genetically-based alternative mating tactics (ANDERSSON, 1994). The presence of two morphs in one population can only be stable if both morphs have the same average fitness (GADGIL, 1972). HEINEMANN & UHL (2000) proved on the basis of carapace measures that *tuberosus* and *gibbosus* are two discontinuous morphs and that the so-called intermediate morphs according to ROBERTS (1987) do not exist in *Oedothorax gibbosus.*

MAELFAIT et al. (1990) proposed, based on only a small number of observations, that the male dimorphism in *Oedothorax gibbosus* is determined by a di-allelic gene only expressed in the male sex. In this model allele G for *gibbosus* is dominant over allele g for *tuberosus* (MAELFAIT et al., 1990). Here, we report data of an elaborate laboratory rearing to test this hypothesis. We also present some other aspects of the development of *Oedothorax gibbosus*.

MATERIAL AND METHODS

The dwarf spiders were caught in the public nature reserve "Het Walenbos" at Tielt-Winge, 30 km north-east of Brussels. Situated on the right bank of the river "de Motte", it is one of the most important river-associated woods of Flanders. The presence of oligo- and mesotrophic alder carrs is typical for "Het Walenbos". The dwarf spiders were caught by hand in such alder carr on October 29, 1998 and on April 9, 1999. The dwarf spiders were placed individually in petri-dishes (3.5 cm diameter and 1 cm height) with a thin bottom of carbonic plaster to allow an observer to see whether the bottom is still humid. This is necessary because of the low resistance of *Oedothorax gibbosus* to desiccation. The petri-dishes were moistened regularly to maintain a relative humidity near 100%. The spiders were kept in a climatic chamber at a temperature of circa 18°C and a photoperiod L:D of 16:8. We opted for 18°C because this resembles the mean wood temperature in summer. Before the second moult spiders were fed at least every two days with four springtails (*Isotoma viridis* among others); after the second moult they were given three fruit flies per day. We reared three generations of descendants starting with 15 males and 15 females from the field. At the start of the first, second and third generation, there were respectively 245, 461 and 223 spiders. To cross one male and one female we placed them in a large petri-dish (5,5 cm diameter and 1.2 cm height). The development of the dwarf spiders, such as the occurrence of the moults and the production of cocoons, was registered accurately using a WILD-binocular dissecting microscope and a cold light source. The development of the dwarf spiders is subdivided into the cocoon phase and the (free) juvenile phase. The cocoon phase is the period between the production of the cocoon and the emergence of the spiders; the (free) juvenile phase is the period between the emergence of the spiders and the last moult.

The following statistical tests were used: t-test with independent variables, Mann-Whitney-U-test, Kolmogorov-Smirnov test, χ^2-test and two-way-ANOVA. We also used the formula of WONNACOTT & WONNACOTT (1990) to calculate the 95% confidence intervals for a proportion:

95% confidence interval = $p \pm 1{,}96 \sqrt{\frac{p(1-p)}{n}}$

(p = proportion, n = sample size)

RESULTS

Mendelian inheritance of male dimorphism

The monogenic di-allelic inheritance model of the male dimorphism, as proposed by MAELFAIT et al. (1990), yields six possible crossing types (Table 1). Especially crossing type 3 shows that the allele G for *gibbosus* is dominant and that the allele g for *tuberosus* is recessive, because this is the only crossing type between a *gibbosus* and a *tuberosus* that results in descendants that all have the *gibbosus* morph. Using the data of the laboratory rearing we have put several family trees together, to investigate if the morph division of the descendants agrees with the inheritance model. To determine the genotype of each female and male we assume that there is no differential mortality in the species *Oedothorax gibbosus* and that a female emerging from a cocoon in which all males have

TABLE 1

The different crossing types with corresponding phenotypes *gibbosus* (*gib*) and *tuberosus* (*tub*) (MAELFAIT et al., 1990)

1.	GG x GG	⟶	GG, GG, GG, GG
	gib *gib*		↓
			gib
2.	GG x Gg	⟶	GG, GG, Gg,Gg
	gib *gib*		↓
			gib
3.	GG x gg	⟶	Gg, Gg, Gg, Gg
	gib *tub*		↓
			gib
4.	Gg x Gg	⟶	GG, Gg, Gg, gg
	gib *gib*		↓
			gib *tub*
5.	Gg x gg	⟶	Gg, Gg, gg, gg
	gib *tub*		↓
			gib *tub*
6.	gg x gg	⟶	gg, gg, gg, gg
	tub *tub*		↓
			tub

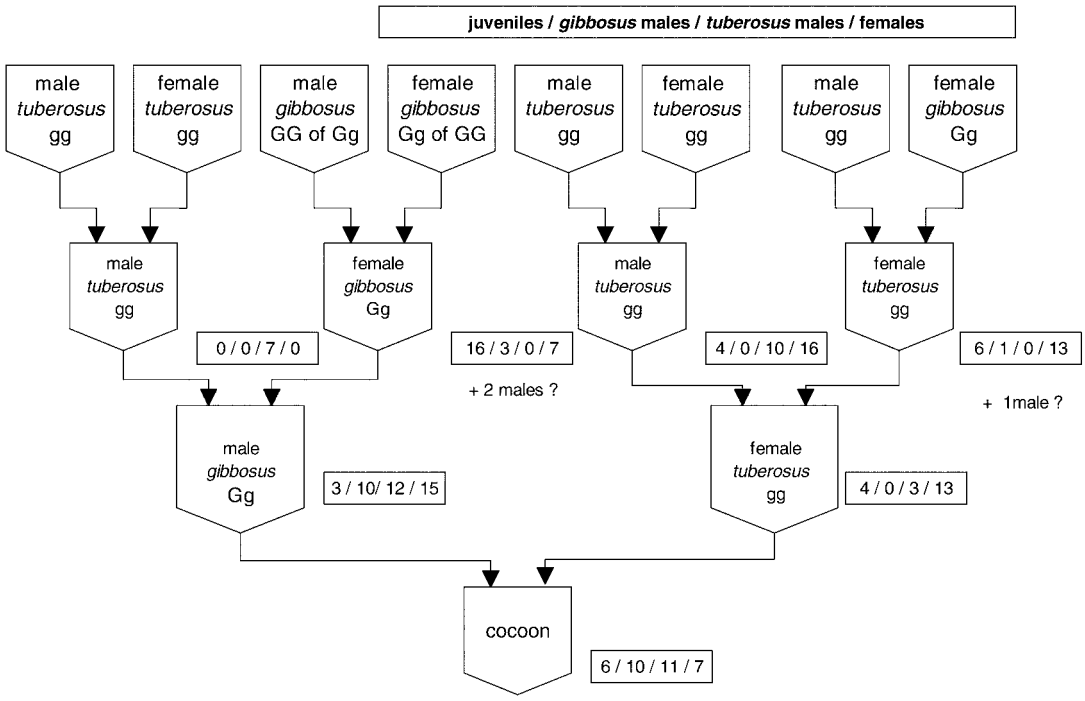

Fig . 1. – An example of a family tree. In this family tree the sex, the code, the morph and the genotype of each spider are indicated. The numbers in the rectangles represent respectively the number of juveniles (= spiders which died before adulthood), the number of *gibbosus* males, the number of *tuberosus* males and the number of females in each cocoon.

the *gibbosus* morph, also has a *gibbosus* genotype; the same applies to the *tuberosus* morph. We obtained ten family trees from the progenitors till the third generation and two family trees till the second generation. Fig. 1 shows an example of such a family tree. Five of the six crossing types appear in these family trees. The first, although self-evident, crossing type (GG x GG → GG, GG, GG, GG) did not appear in any family tree. Especially crossing types 4, 5 and 6 are in majority. All family trees were in agreement with the postulated di-allelic monogenic inheritance of male dimorphism by MAELFAIT et al. (1990).

The fifth moult

Approximately 5% of the dwarf spiders moulted five times in the laboratory. The spiders that moulted five times needed this last moult effectively to reach adulthood. The frequency of moulting five times was approximately equal in males and females; 11 of 165 males and 20 of 291 females moulted five times. According to the χ^2-test these proportions are not significantly different (df = 1, p = 0,937). Significantly more *tuberosus* males than *gibbosus* males moulted five times (χ^2-test: df = 1, p = 0,014); ten of 87 *tuberosus* males and one of 78 *gibbosus* males moulted five times. The number of moults has an important effect on the cocoon phase. This could not be investigated in the first generation of dwarf spiders because in this generation there was no single case of a fifth moult. In the third generation the cocoon phase was significantly shorter in the spiders that moulted five times than in the spiders that moulted four times (t-test: $n_{5moults}$ = 12, $n_{4moults}$ = 73, df = 83, p = 0,002; U-test: $n_{5moults}$ = 12, $n_{4moults}$ = 73, U = 223, Z = -3,05, p = 0,002). In the spiders with five moults the mean cocoon phase was 16 ± 1 days; on the other hand this was 18 ± 2 days in the spiders with four moults. There was also a significant effect of the number of moults on the cocoon phase according to the t-test in the second generation (t-test: $n_{5moults}$ = 18, $n_{4moults}$ = 260, df = 276, p = 0,032), although this effect is not significant according to the U-test (U-test: $n_{5moults}$ = 18, $n_{4moults}$ = 260, u = 1551, Z = -0,64, p = 0,523).

The juvenile development

The ANOVA test analysis of the effect of the (male) spider morph and the generation on the juvenile phase, yielded the following results. The juvenile phase of *gibbosus* was, in generations 1 and 2 (df Effect = 1, df Error = 351), significantly longer than the juvenile phase of *tuberosus* (F= 11,106; p= 0,001). The same result was obtained for generations 2 and 3 (df Effect = 1, df Error = 103) and for generations 1 and 3 (df Effect = 1, df Error = 48). The t-test as well as the U-test confirms this result. Table 2 shows the mean juvenile phase of each generation. The ANOVA test that analyses the effect of the crossing type (crossing type 1, 4 and 6) and the sex on the juvenile phase in generation 2, shows that the crossing type, as was to be expected, had a significant effect on the juvenile phase (df Effect = 2, df Error = 205, F = 8,8, p = 0,0002). Only the three most com-

monly occurring crossing types were used for this test, and the spiders coming from crossing types that yielded more *gibbosus* offspring had a significant longer juvenile phase (fig. 2). This test also shows that females had a significant longer juvenile phase than did males (df Effect = 1, df Error = 205, F = 12,0, p = 0,0006) and that there was no significant interaction between the effect of crossing type and sex on the juvenile phase (df Effect = 2, df Error = 205, F = 1, 9, p = 0,16). This last observation is very important because it means that the effect on the juvenile phase also holds for the females although the genotype of dimorphism is not expressed in their phenotype. So-called '*gibbosus* females', this means females that have a genotype with at least one G, have also a significantly longer juvenile phase.

TABLE 2

Survey of the mean values ± standard deviation of cocoon phase and juvenile phase in each generation separately and in all generations together

generation	cocoon phase	juvenile phase
1	18 ± 2	42 ± 4
2	15 ± 4	50 ± 6
3	18 ± 2	50 ± 8
1+2+3	17 ± 3	44 ± 6

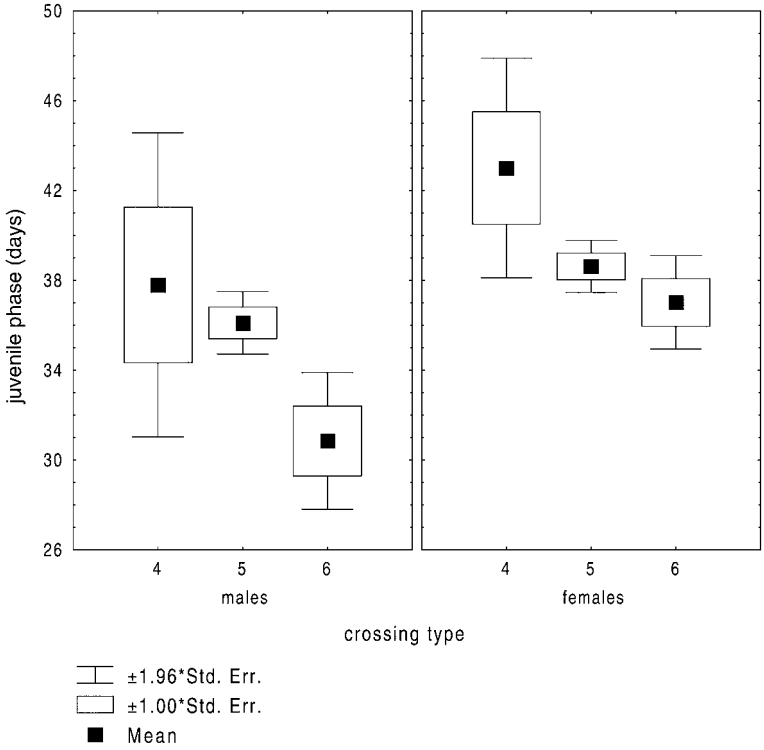

Fig. 2. – The effect of crossing type and sex on the juvenile phase in generation 2. Each framework represents a sex, each box-and-whicker a crossing type. The juvenile phase is significantly longer in the spiders of crossing type 4 and 5 (*gib*) than those of crossing type 6 (*tub*). The juvenile phase of the spiders of crossing type 4 (2x G) is almost significant longer than those of crossing type 4 (1x G).

Sex ratio distortion in favour of the female sex

In both field catches of the spiders, the female sex predominated; in the first catch the percentage of females was 66%, in the second catch 61%. So the sex ratio in the population of "Het Walenbos" is approximately 1 to 2; this means theoretically that for each male there are two females available. This sex ratio was also maintained in the dwarf spiders of the three laboratory generations: in generations 1, 2 and 3 the sex ratios were respectively 47 to 98, 107 to 196 and 58 to 95. According to the χ^2-test the sex ratios of the three different generations are not significantly different (df = 2, p = 0,614). Table 3 shows the sex ratio of the two most commonly occurring crossing types in the laboratory rearing. In crossing type 5 a distinction is made between the situation that Gg or gg is the paternal or the maternal genotype. If gg is the maternal genotype, the sex ratio is approximately fifty-fifty. On the other hand the sex ratio is shifted in favour of the female sex if gg is the paternal genotype. In crossing type 6 gg is the paternal as well

TABLE 3

Sex ratio of the two most frequent crossing types in the laboratory rearing. In crossing type 5 a distinction is made between the situation that Gg or gg is the paternal or maternal genotype *The sex ratio is here expressed as ratio of total number of male offspring divided by the total number of offspring for each crossing type (families = number of observed families for each crossing type, males = sum of males of all families for each crossing type, females = sum of females of all families for each crossing type)

crossing type	families	male genotype	female genotype	males	females	sex ratio*
5	8	Gg	gg	48	53	48 %
5	12	gg	Gg	65	115	36 %
6	18	gg	gg	52	110	32 %

as the maternal genotype. The sex ratio in this crossing type is completely shifted in favour of the female sex (Table 3). According to the χ^2-test there is a significant difference between those three sex ratios (df = 2, p = 0,039). The confidence intervals of the sex ratio for every situation overlap partly, but if the paternal genotype is gg and in the case of crossing type gg x gg, the confidence interval is situated under the fifty-fifty sex division (Fig. 3).

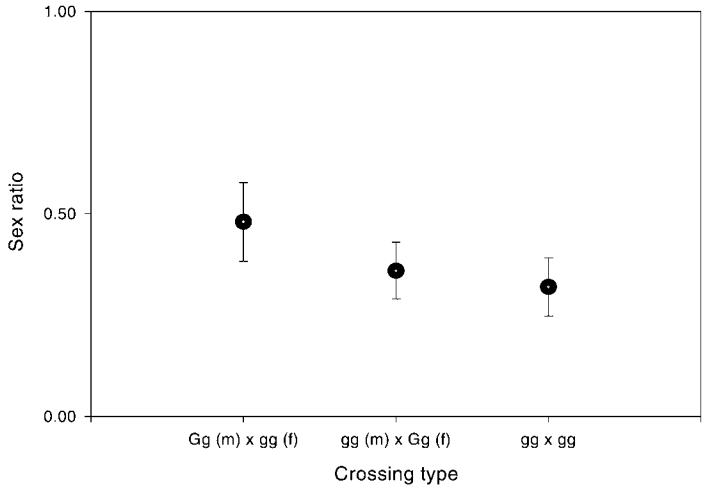

Fig. 3. – The confidence intervals of the sex ratios of the two most occurring crossing types in the laboratory rearing. Dots indicate the ratio of total number of male offspring divided by the total number of offspring for each crossing type. Error bars indicate 95% confidence intervals calculated with the formula of WONNACOTT & WONNACOTT (1990).

DISCUSSION

The monogenic, di-allelic inheritance model of the male dimorphism in the dwarf spider *Oedothorax gibbosus* is confirmed by the analysis of the different family trees. Five of the six possible crossing types appeared in the laboratory rearing. The fact that the very evident crossing type 1 (GG x GG ➔ GG, GG, GG, GG) is absent in the rearing, is probably due to chance. The fact that the longer juvenile phase also appears in females with a *gibbosus* father, points out that the genotype of dimorphism also influences the female sex, although this genotype is not expressed phenotypically in females. A great disadvantage during the research was high mortality in the dwarf spiders. This may have caused a small inaccuracy in the results.

The representatives of the Linyphiidae are characterised by a great constancy of four moults, although there have been exceptions found: *Stemonyphantes lineatus* moults nearly always five times and *Floronia bucculenta* mostly moults also five times (SCHAEFER, 1987). Both species belong to the subfamily of the Linyphiinae, which are characterised by larger sized spiders. *Oedothorax gibbosus,* however, is a representative of the subfamily Erigoninae which contains smaller dwarf spiders. It is surprising that such a little dwarf spider as *Oedothorax gibbosus* sometimes needs an additional moult because according to ROBERTS (1995) smaller spiders moult fewer times than do larger spiders, but SCHAEFER (1987) already points out that there is no clear correlation between the size of the spider and the number of moults. In *Oedothorax gibbosus* the fifth moult occurs only occasionally, but this fifth moult is necessary to reach adulthood and maturity. The dwarf spiders that moult five times also hatch significantly earlier, and this probably makes an additional moult necessary. This is the first time that an exception to the normal number of four moults in dwarf spiders of the subfamily Erigoninae has been observed.

The juvenile phase of *gibbosus* males is significantly longer than that of *tuberosus* males. This is perhaps necessary for the production of the hunch and the hairy groove, which may need a high energy investment. The shorter development rate is a possible advantage for *tuberosus*. When temperature is high the development rate increases and the development time decreases. One can demonstrate on the basis of the Q10 rule that the advantage of *tuberosus* males would be greater if it is colder. Q10 is the factor by which a physiological function increases as response to a temperature rise of 10 °C (PROSSER, 1973). The factor Q10 mostly varies from 2 to 3 (PULZ, 1987). In other words if the temperature increases 10 °C the development rate, for example, increases by a factor of 2 to 3. Let's suppose that the factor Q10 for the development in *Oedothorax gibbosus* is 3. When it is 18 °C the mean juvenile phase of *gibbosus* males and *tuberosus* males is respectively 44 and 37 days. This is a difference in juvenile development time of 7 days. If the temperature would increase 5 °C (23 °C), the development rate would increase by a factor of 1.5 and the development time would be decreased by a factor of 1.5. The difference in juvenile development time between both morphs would then only be 4 days. There is thus a large overlap between the appearance of mature *tuberosus* and *gibbosus* males. Because of the presence of the hairy groove and the dominance of *gibbosus,* a *gibbosus* male has an advantage from the moment that it can copulate; therefore the *tuberosus* male would have little advantage with a lead of 4 days. If the temperature would decrease 5 °C (13 °C) the development rate would decrease by a factor of 1.5 and the development time would increase by a factor of 1.5. The difference in juvenile development time would be 10 days. *Tuberosus* males would have a greater lead and could fertilise the mature females earlier. This scenario is purely hypothetical and has to be further investigated.

Normally one would expect that there are as many males as females in a population, because according to FISHER (1930) natural selection promotes a fifty-fifty sex ratio. In "Het Walenbos" the sex ratio is 1 to 2 and one can deduce from the results that the *tuberosus* morph probably

can compensate the genetic dominance of the *gibbosus* morph by a sex ratio distortion in favour of the female sex. The mechanism of this is still unknown, but there are many known examples from the literature in which parents influence the sex ratio of their offspring (TRIVERS & WILLARD, 1973; CLARKE, 1978; CLUTTON-BROCK et al., 1984; SUGIARA, 1994; SVENSSON & NILSSON, 1996; CAZEMAJOR et al., 1997; KOMDEUR et al., 1997; BRADBURY & BLAKEY, 1998; KILNER, 1998; WERREN & BEUKEBOOM, 1998).

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Variability of the bioelectric field of the catfish *Ictalurus nebulosus* (Le Sueur, 1819; Pisces, Teleostei, Siluriformes)

Robert C. Peters, Cor G.J. van Honk and Franklin Bretschneider

Department of Comparative Physiology, Neuroethology Group, Utrecht University, Padualaan 8, NL-3584 CG Utrecht, The Netherlands

ABSTRACT. The electric dc-field of *Ictalurus nebulosus* (Teleostei, Siluriformes) was recorded by having two specimens swim through a silk screen cylinder at 5 cm distance from two recording electrodes, during an observation period of three months.

The dc-field was usually rather stable; on average potential differences of 37 and 55 μV in water with resistivities of 33 and 48 ohm.m respectively were recorded. From time to time the field strength suddenly increased by a factor of 10, after which the field returned to the original value within about 15 min. Increases of field strength could also be evoked by presenting food, "beef juice", and by approaching the tank.

We propose that the recorded changes in bioelectric field strength reveal a particular, perhaps stress- or arousal-related, physiological condition, and that they are sufficiently strong to be perceived by electrosensitive conspecifics.

KEY WORDS: *Ictalurus nebulosus*, lateral line, electroreception, electric field, bioelectric field, transdermal potential, electrocommunication.

INTRODUCTION

Ampullary electroreceptor organs in catfish respond mainly to signals with spectral components between dc and 100 Hz. (BULLOCK & HEILIGENBERG, 1986; KALMIJN, 1974; KRAMER, 1996; MOLLER, 1995). Such low frequency components are generally not produced by specialized electric organs or muscle activity, but are rather a by-product of metabolic processes in organisms, and geophysical processes of the aquatic environment (PETERS & BRETSCHNEIDER, 1972). One of the sources of electrical stimuli to ampullary electroreceptor organs is the fish itself (BUTSUK & BESSONOV, 1981; PETERS, 1973; PETERS & MEEK, 1973; ROTH, 1969; ROTH, 1972). It has been demonstrated for instance that freshwater catfish are surrounded by stationary electrical dc-fields upon which ac-components, related with respiration, are superimposed. Such fields have been demonstrated in many species of aquatic organisms (KALMIJN, 1972). They can reveal the presence of an individual to other electrosensitive species. The aim of the present paper is to investigate how stable such fields are and if changes are related to, for instance, feeding.

Corresponding author: R.C. Peters, e-mail: r.c.peters@bio.uu.nl

MATERIAL AND METHODS

Animals

All tests were performed on two specimens of the freshwater catfish *Ictalurus nebulosus.* The fish remained for about three months in the recording setup, which was a part of full glass tanks of 150x60x60 cm. During their confinement they were fed on minced beef and occasionally maggots and pellets. The experiments were performed at room temperature, which was about 14 or 20 °C. The potentials of two specimens were recorded at two different locations during 590 hours in all.

The specimen at location 'A' was kept in a light:dark regime of L:D = 11.5:12.5, at 20ºC, with water resistivity of 33 Ohm.m. Recordings were made during 330 hours.

The fish at location 'B' was kept in a light:dark regime L:D = 12:12 at 14ºC with water resistivity of 48 Ohm.m. Recordings were made during 260 hours.

Electrodes and equipment, electric field recording

The electric field was recorded by means of sintered silver/silver chloride electrodes, equilibrated for about two months in fresh water, mounted in a pvc cylinder to keep them free from unwanted turbulence, and shielded from light in order to reduce light induced potential changes. PAR 113B differential pre-amplifiers were used to boost the signals. The potentials were recorded on paper chart recorders to provide a clear overview on the course of the field changes. In order to eliminate the effects of electrode drift we made ac-recordings at a bandwidth of $0.1<f<10$ Hz and $0.03<f<30$ Hz at locations 'A' and 'B' respectively, by having the fish pass a set of fixed electrodes**.** By passing the electrodes, the dc-field of the fish causes an ac-wave at the site of the electrodes which can be recorded quite accurately. The 'recording device' was a silk screen cylinder connecting two compartments of the aquarium. The cylinder was 15 cm long, had a diameter of 8 cm, and did not distort the expansion of the electric field. Below the cylinder, at 5 cm distance, two recording electrodes were fixed 7 cm apart. The differential recording allows the detection of the swimming direction of the fish. The whole tank was illuminated from above by a light bulb of 100 W.

RESULTS

Control

In order to test the setup for sensitivity to mechanical turbulence, a silicon rubber dummy fish was pushed through the recording cylinder at a speed of 30 cm/s, which caused rather rough water motion. This resulted in potentials of less than 10 μV.

Stability and variability

At location 'A' the average potential change of 7565 passages during 330 hours was 37 μV, with standard deviation 28 μV, and S.E.M. 0.3 μV.

At location 'B' 815 passages during 44 hours gave an average potential change of 55 μV, with standard deviation 0.9 μV, and S.E.M. 0.03 μV. In a second recording session 4562 passages were counted during 216 hours. The average potential change was 84 μV, with standard deviation 59 μV, and S.E.M. 0.9 μV.

In general we observed that, if no food or novel stimuli were presented , the form and strength of the bioelectric field was stable (see Fig. 1A).

Administration of food and stress

During the recording period we observed 19 sudden increases in field strength. The recorded field strength went up by a factor of 10, and returned to the original level in 15 min or more. In nine of these cases the increase could not be related to any apparent cause (see Fig. 1B). In one case the increase could be related to an observer approaching the tank (Fig. 1C). The remaining increases were due to the administration of food or 'beef juice'. In five cases, feeding the fish caused an increase of the bioelectric field indeed, as well as an increase in locomotor activity. Putting pieces of minced meat into the water almost immediately caused an increase of the field strength, which could last for more than 15 min. In four cases the increase in field strength was caused by putting a few drops of 'beef juice' into the water (Fig. 1C). Taking a fish out of the tank and putting it back later induced a similar increase in field strength to feeding. Withholding food for one week decreased the bioelectric field to values as low as 10 μV.

DISCUSSION AND CONCLUSION

The many thousands of recordings made during hundreds of hours in these two fish demonstrate that the bioelectric field of *Ictalurus nebulosus* is stable for a considerable time. From time to time this stable field increases either spontaneously, i.e. without known cause, or as the result of novel stimuli such as the administration of food. Calculations showed that the recorded field patterns can be simulated by a head-negative and tail-positive dipole source, with a current of 1 μA, in water with a resistivity of 33 ohm.m, with the current sink and current source 7 cm apart. The recorded potentials depend on the swimming speed of the fish, the bandwidth of the preamplifier, the distance between recording electrodes and fish, the span between the recording electrodes, and the conductivity of the cylinder. A more precise description of the field form and the field sources can not be accounted for at this moment.

The interesting finding of the present study is that administration of food causes increases in field strengths that strongly resemble the 'spontaneous changes'. The speed of the change suggests neural control. The time course of the subsequent recovery points to the involvement of some humoral component. These sudden increases of the bioelectric field could not be missed by other, passing, electrosensitive catfish. The field change is a sufficiently conspicuous stimulus to contain a 'message' to other electrosensitive conspecifics. If all aquatic organisms show similar increases in electric field strength when they have fed, they may become more conspicuous to their electrosensitive predators as well.

The conclusion is that the bioelectric field of catfish is, as a rule, rather stable, but that it can increase in strength tenfold either spontaneously, induced by the administration of food, or by alarming events. The sudden increase lasts for about 15 minutes. The precise origin of the field change is not known. Most likely neural or hormonal control of epithelial ion pumps is involved. The changes of the bioelectric field are so conspicuous and characteristic that most likely they may be considered as signals carrying information to other conspecifics or other electrosensitive species.

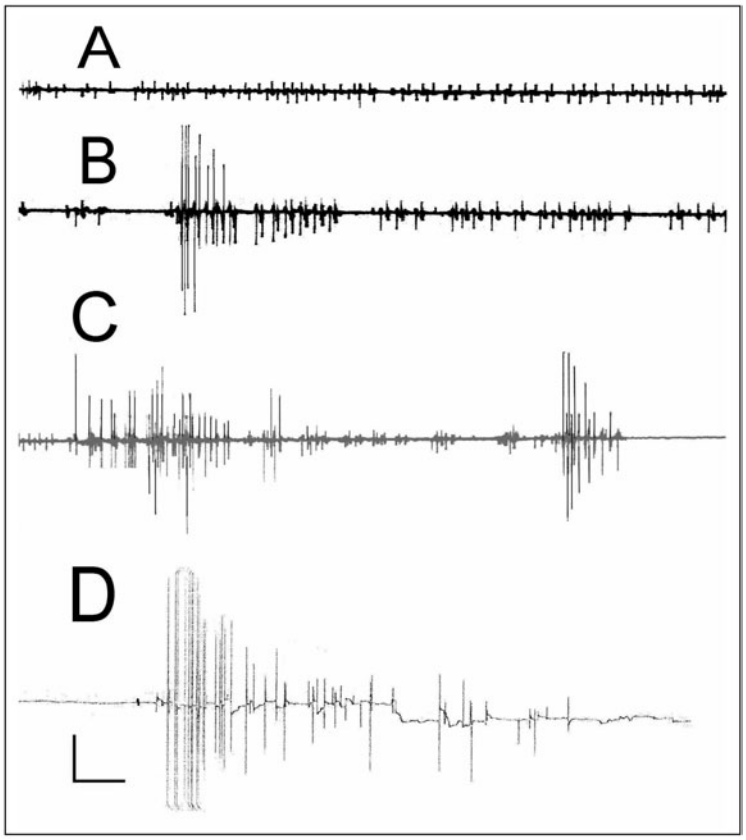

Fig. 1. – Examples of paper chart recordings of potential changes caused by a catfish, *Ictalurus nebulosus*, passing a set of electrodes spaced 7 cm apart at a distance of 5 cm in freshwater at room temperature. The original recordings were scanned after which the background was removed.

A: Location 'A'. Regular to-and-fro passages reveal a stable electric field. The recorded potential changes are on average 55 μV. Scale bars 250 μV and 2 min.

B: The same setup as under A. Here the field strength suddenly increases without any known cause. The recording was made on thermosensitive paper, which did not always receive enough heat to record the passages properly.

C: The same set-up as under A and B. The field strength increases twice. The first time after injection of 'beef juice' in the water; the second time after an observer entered the room and approached the tank.

D: Location 'B'. Sudden increase in field strength in the second setup. This recording was made with a carbon copy recording system. Scale bars 33 μV, 5 min.

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Habitat and territory segregation within Sylviine warblers of the Flemish coastal dunes

Dries Bonte[1], **Sam Provoost**[2] **and Maurice Hoffmann**[2]

[1] **University of Ghent, Department of Biology, Laboratory of Animal Ecology, Zoogeography and Nature Conservation, K.L. Ledeganckstraat 35, B-9000 Ghent**
[2] **Institute of Nature Conservation, Kliniekstraat 25, B-1070 Brussels**

ABSTRACT. Sylviine warblers are abundant breeding birds in the Flemish coastal dunes. Although their habitat preferences are clearly different, habitat overlap can occur. Their habitat preferences can largely be explained by the overall territory-specific vegetation structure (all components included in the discriminant analysis). The transition from woodland to scrubs and from scrubland to short grasslands explains 92% of the total variance within the species' habitat characteristics. Species of the different genera (*Sylvia, Phylloscopus, Acrocephalus, Locustella*) show a large amount of habitat overlap. Within the *Phylloscopus* and *Sylvia*-genus, only the Lesser Whitethroat *S. curruca* and the Whitethroat *S. communis* use the same breeding habitat. Although the other congeneric species show distinct habitat characteristics, a priori classification cannot predict territory occupancy without errors: only 78.3% of the two *Phylloscopus*-territories and 58.3% of the *Sylvia*-territories were correctly classified. This indicates a certain amount of territory settlement in non-typical habitats, where competition can occur between sister species.

All possible interactions between congenerics were studied by comparing the expected (based on the total of wrong classifications in the typical habitat of the other) and the observed coexistence. The number of wrong classifications could only be explained by real coexistence in the species pair *S. borin-S. curruca*. Interactions between all other congeneric species pairs were asymmetrical, resulting in distinct territory occupancy with one dominant species. *S. borin* was always the dominant species, whereas *S. communis* was never dominant within the possible interactions. In general, species typical for higher vegetation were dominant (with the exception of the species pair *S. atricapilla-S. borin*). Direct and song aggressiveness are probably the driving forces for the observed territory segregation. Our results confirm and supplement the findings of CODY (1978), who studied similar habitat and territory segregation in Sylviine Warblers in England, Southern Sweden and Sardinia.

KEY WORDS: *Acrocephalus*, *Locustella*, *Phylloscopus*, *Sylvia*, interspecific interactions, habitat characteristics, vegetation, discriminant function analysis, Geographic Information System.

INTRODUCTION

During 1998, breeding birds were inventoried in the Flemish coastal dunes within the framework of a monitoring project, financed by the Flemish government, Nature Division AMINAL (Life-project – ICCI Integral Coastal Conservation Initiative). The aim of this project was to describe the breeding bird species composition and their landscape ecological relationships as a tool for the evaluation of the applied nature management (BONTE et al. 1998).

Corresponding author: D. Bonte, e-mail: dries.bonte@rug.ac.be

The inventory yielded a total of 78 species with a total of 4455 territories. The most common species were Dunnock *Prunella modularis*, Wood Pigeon *Columbo palumbus*, Whitethroat *Sylvia communis*, Blackbird *Turdus merula* and Willow Warbler *Phylloscopus trochilus*. Also nine Flemish Red List species were noted as breeding birds.

Sylviine-warblers (Marsh Warbler *Acrocephalus palustris,* Grasshopper Warbler *Locustella naevia,* Chiffchaff *Phylloscopus collybita,*Willow Warbler *P. trochilus,* Blackcap *Sylvia atricapilla,* Garden Warbler *S. borin,* Whitethroat *S. communis and* Lesser Whitethroat *S. curruca*) were particularly abundant, and their territories could be easily mapped by applying common bird census

techniques. An initial analysis (BONTE et al., 2001) indicated that all Sylviine warblers are restricted to half open or closed dune scrub landscapes. Their presence in a common environment is very interesting because it enables us to study their specific habitat preferences and the potential niche overlap or coexistence based on the detailed vegetation description of their territories.

Of the mentioned Sylviine warblers, only *L. naevia* is a rather rare species in Flanders (200-500 pairs, with more than 10% of the total population along the Flemish coastal dunes: DEVOS & ANSELIN, 1999). All other species are common and occur in a variety of woody habitats (including gardens and woodlands). They are all insectivorous birds and typical summer guests in North-Western Europe. Chiffchaffs *P. collybita* and Blackcaps *S. atricapilla* are partial migrants with part of the population annually hibernating in our temperate regions. In the south of Europe they are year-round residents. The other species winter in northern or sub-Saharan Africa . Many of the Sylviine warblers are characterised by similar habitats and food preferences in the winter-grounds and tend to segregate their winter habitats in a similar way as in the breeding quarters (CODY, 1985).

Sylviine warblers are territorially aggressive during the breeding season, and most of them seem to be opportunistically polygynous. Many authors have reported interspecific territoriality in Sylviine warblers (CRAMP & BROOKS, 1992). CODY (1978) documented this phenomenon between Willow Warblers *P. trochilus* and Chiffchaffs *P. collybita* in England; *Hippolais* species are interspecifically territorial when their home ranges overlap in France (FERRY & DESCHAINTRE, 1976 in: CRAMP & BROOKS, 1992). CODY (1985) recorded interspecific territoriality in congeneric species of the genus *Sylvia* in Southern Sweden, England and Sardinia. The latter also states that interspecific territoriality is a rare phenomenon between *Sylviine* warblers of different genera, although some evidence exists of interactions between *Hippolais sibilatrix* and *Sylvia atricapilla* (CRIVELLI & BLANDIN, 1977). CODY (1978) stated further that these interspecific interactions are presumably effected through interspecific reactions to territorial songs (song convergence).

CODY (1985) gives a detailed overview of his results on habitat segregation and interspecific interactions within *Sylviine* warblers in Sweden, England and Sardinia. In this contribution we firstly studied whether habitat segregation of *Sylviine* warblers is similar to that in other regions in Europe. Secondly we investigated whether interspecific interactions occur in the Flemish coastal dunes as well and if so, in what way they differ from those mentioned in other studies.

STUDY AREA

The study was undertaken in the coastal dunes between Dunkerque (North of France) and Nieuwpoort (Belgium). Eight dune sites were selected in order to cover a substantial portion of the landscape-ecological range within young dunes. The sites are situated in the Perroquet (Bray Dunes, France; 225 ha), the Westhoek (340 ha), Houtsaegerduinen (80 ha) (De Panne, Belgium), the Noordduinen (45 ha), Doornpanne (160 ha) and parts of the Ter Yde dune complex (110 ha) (Koksijde, Belgium).

These aeolian dunes are characterised by large dune slacks and distinct parabola-shaped ridges. Some mobile dunes are almost without vegetation. The wandering dunes in the Westhoek, for example, consist of nearly 100 ha of drifting sand, leaving large dune slacks on the lee side. Front dunes and smaller mobile dunes are covered with *Ammophila arenaria* (Marram grass). Fixed dune ridges primarily develop into grey dunes, mainly dominated by *Tortula ruralis* var. *ruraliformis* or *Hypnum cupressiforme* var. *lacunosum. Hippophae rhamnoides* scrub (Sea-buckthorn) dominates the next successional stage, and climax vegetation in dry dunes consists of woodland with *Quercus robur, Fraxinus excelsior* and *Acer pseudoplatanus*.

In dune slacks succession occurs significantly faster. Within a decade bare sand can turn into scrub or even woodland. Large dune slacks can be covered with various types of scrubland depending on elevation and successional stage. Dominant species are mainly *Hippophae rhamnoides, Salix repens* and *Ligustrum vulgare* but *Sambucus nigra, Crataegus monogyna* and *Rosa* spp. can be abundant as well. These species contribute to the structural diversity of the scrub. Besides trends towards woodland development, scrub can degenerate and be replaced by species-poor *Calamagrostis epigejos* stands.

Former livestock grazing or actual nature management can keep vegetation in a grassland or marshland stage. Botanically these vegetations can be very rich with species such as *Thesium humifusum, Helianthemum nummularium* and *Potentilla neumanniana* in dry grassland and *Epipactis palustris, Centaurium littorale* and *Parnassia palustris* in wet dune slacks.

MATERIAL AND METHODS

Field methods

In 1998, we carried out a census of Sylviine warblers in the Flemish coastal dunes (BONTE et al., 2001), using the territory mapping method (common bird census, CBC) as described by HUSTINGS et al. (1985). Common bird census is the only reliable method to obtain detailed information about absolute breeding bird densities and territory distribution in a particular area. A total of ten mapping visits were made during the breeding season (March to July).

Detailed vegetation maps of the larger, non-urbanised dune areas, which were not available (Westhoek and Perroquet), were produced by a stereoscopical interpretation of aerial photographs with scale 1:2000. The result-

ing contour maps were digitised with the GIS package Genamap 6.2, checked in the field and coded for vegetation composition with the units proposed and used by PROVOOST & HOFFMANN (1996). Detailed digital vegetation maps of the Doornpanne (KUIJKEN et al., 1993), Ter Yde (HOFFMANN et al., 1998) and Lombardsijde (HOFFMANN et al., 1996) were available at the Institute of Nature Conservation (Brussels). The following vegetation types were lumped into structurally similar units: dune woodland, mixed shrubs (*Hippophae rhamnoides* with *Rosa* species and *Crataegus monogyna*). *Ligustrum vulgare* dominated shrub, *Salix repens* dominated shrubs, *Hippophae rhamnoides-Sambucus nigra* shrubs, tall grasslands dominated by *Calamagrostis epigejos*, short rabbit grazed pastures, grey dunes and blond dunes with Marram grass (*Ammophila arenaria*).

Data Analyses

We used the interpretation criteria of HUSTINGS et al. (1985) to construct the specific territory maps. These maps were digitised within a GIS (Genamap 6.2). The simplified vegetation and territory maps were imported in Arcview 5.1, by means of which overlays were made. In this way, specific vegetation descriptions were obtained for each territory.

Differentiation in habitat structure (i.e. vegetation composition) was studied by a forward discriminant analysis (Statistica 5.1) for all Sylviine warblers, and specifically for the congeneric *Sylvia* and *Phylloscopus* species, for all species together, and for the different studied species pairs.

The discriminant analyses were used to determine which vegetation types were the best predictors for the habitat segregation. Habitat segregation was studied by canonical plots of the discriminant functions, with 95% confidence ellipses around the species' means and Mahalanobis distances. The significance levels of the Pearson correlation were calculated following JERROLD (1996). For each species combination, *a priori* classifications revealed the number of correctly classified territories and the amount of occupied territories, in the typical habitat for the congeneric species. The wrongly classified territories should be in reality be the result of common habitat use because of coexistence in the field or from common habitat use without territory overlap as a result of direct interspecific interactions, where one species excludes the other.

The percentage of predicted common habitat use (predicted coexistence) was compared with the observed percentage of coexistence (we assumed coexistence when the territories overlapped) by the application of a χ^2-test (JERROLD, 1996). When the observed proportion of coexistence does not differ from the expected, common habitat use is the result of real coexistence. If the observed proportion of territory overlap is significantly lower than the expected, common habitat use is accompanied by interspecific territorial interactions and thus by territory segregation. These interactions are either symmetrical when the two species equally occupy the typical territory of each other, or asymmetrical in the case when one dominant species occupies more territories in the sister species typical habitat. These differences were again tested by χ^2-tests for deviations from equal proportions .

RESULTS

Habitat overlap between *Sylviine* warblers

In the study area, a total of 1296 territories of the eight Sylviine species were recorded (Table 1): *Sylvia communis* and *Phylloscopus trochilus* were the most abundant species (resp. 331 and 324 territories); *Acrocephalus palustris* was the rarest included Warbler (22 territories).

TABLE 1

Total number of territories in the study area per species

Species	Total number of territories
Acrocephalus palustris Bechstein, 1798	22
Locustella naevia Boddaert, 1783	55
Phylloscopus collybita Vieillot, 1817	254
Phylloscopus trochilus Linnaeus 1758	324
Sylvia atricapilla Linnaeus 1758	176
Sylvia borin Boddaert, 1783	65
Sylvia communis Latham, 1787	331
Sylvia curruca Linnaeus 1758	69
Total	1296

All variables were included in the forward discriminant function analysis (Table 2). Although the overall discriminating power is low (36.88 % correctly classified), the

TABLE 2

Vegetation components included in the discriminant function analysis (Wilks' Lambda= 0.654, F (77,7665)=7.293, $p<0.0001$), based on all species. Bold: significant contribution to the overall discrimination.

Vegetation type	Wilks' Lambda	p-level	R^2
Grey dunes	0.662	0.063	0.045
Ligustrum vulgare shrub	0.659	0.324	0.009
Marram dunes	0.659	0.326	0.099
Mixed shrub	0.660	0.141	0.511
Salix repens-shrub	0.657	0.589	0.051
Sambus nigra-shrub	0.657	0.612	0.279
Sandy dune slack	0.656	0.826	0.046
Short grassland	**0.664**	**0.013**	**0.327**
Tall grassland	0.659	0.366	0.077
Woodland	**0.817**	**0.000**	**0.397**
Young dune slack	0.657	0.631	0.051

analysis was highly significant, indicating that the species' territories can be discriminated on the basis of their vegetation composition. Of all included vegetation types, only two contributed significantly to the overall discrimination: the proportions of woodland and short grassland within the territories.

Of the seven calculated canonical axes, only the first two contribute significantly to the total amount of variation (Table 3). Although the first axis contributes 86.4 % and the second another 5.6 %, the eigenvalue of the second axis is very low. The first canonical axis correlates significantly (Table 3) with the proportion of woodland (negatively) and different types of scrub (positively) within the territories. This axis is also positively, but less significantly, correlated with open sandy dunes and short vegetation. The second axis correlates negatively (Table 3) with the amount of different types of scrubland, and positively with short vegetation and open dune (particularly with short grasslands).

The plot of the discriminant functions with species' habitat means surrounded with 95% confidence ellipses (Fig. 1) visualises the habitat segregation: congeneric *Sylvia* and *Phylloscopus* species segregate completely. A certain habitat overlap exists between the species pair *P. collybita* and *S. atricapilla*. *Acrocephalus palustris* and *S. curruca* are characterised by a certain amount of habitat overlap, while the latter shares a common habitat with both *Locustella naevia* and *Phylloscopus trochilus*. Territories of the latter species pair overlap consistently.

Taking into account the canonical correlation, *P. collybita* and *S. atricapilla* can be categorised as dune woodland-preferring species and *S. borin* as an intermediate species between scrubs and woodland. All other *Sylviine* warblers prefer scrub. Of these, *S. communis* and *L. naevia* are associated with grassland-shrub mosaics, while *S. curruca* and *A. palustris* apparently prefer homogeneous scrub.

In contrast to the results of the canonical ordination, the Mahalanobis distances (Table 4) indicate significant habitat segregation only between woodland associated species (*P. collybita* and *S. atricapilla*) and the other *Sylviine* warblers. *S. borin* shows no habitat segregation with *A. palustris* and a low one with *S. atricapilla*.

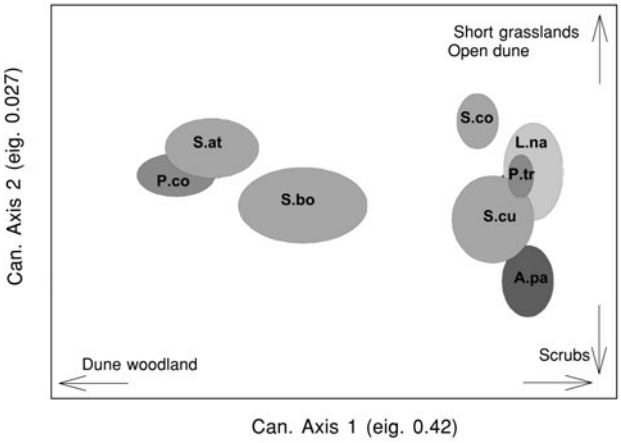

Fig. 1. – Discriminant ordination plot of the species' territories (means and 95% confidence ellipses) with eigenvalues (eig.) based on the vegetation composition within the territories. Species abbreviations: A.pa: *Acrocephalus palustris*, L. na: *Locustella naevia*; P. tr: *Phylloscopus trochilus*; P. co: *Phyllsocopus collybita*; S. at: *Sylvia atricapilla*; S. bo: *Sylvia borin*; S.co: *Sylvia communis*; S. cu: *Sylvia curruca.*

TABLE 3

Correlation coefficients and canonical analysis results of the vegetation characteristics with the seven calculated canonical roots (Spearman correlations, n=1296; **bold: p<0.001**; underlined: p≤0.01).

Vegetation type	**Root 1**	**Root 2**	Root 3	Root 4	Root 5	Root 6	Root 7
Grey dunes	0.037	**0.146**	0.627	0.035	0.092	-0.221	0.407
Ligustrum vulgare shrub	-0.004	**-0.146**	0.418	-0.075	0.336	0.321	0.274
Marram dunes	0.096	**0.222**	0.388	-0.137	-0.436	0.097	-0.144
Mixed shrubs	**0.247**	**-0.600**	0.112	0.501	-0.110	0.106	-0.335
Salix repens-shrub	**0.140**	0.059	-0.060	-0.568	0.054	0.060	0.143
Sambucus nigra-shrub	**0.233**	**-0.146**	0.042	-0.436	-0.255	-0.625	0.077
Sandy dune slack	**0.116**	0.088	-0.139	-0.537	-0.009	0.108	-0.215
Short grasslands	0.095	**0.817**	-0.011	0.242	0.228	-0.118	-0.272
Tall grassland	-0.005	0.021	-0.440	0.294	-0.022	-0.307	0.534
Woodland	**-0.973**	0.002	-0.006	-0.208	0.000	-0.036	0.013
Young dune slack	0.037	**0.164**	-0.102	0.014	-0.682	0.431	0.377
Eigenvalue	0.429	0.028	0.023	0.009	0.004	0.002	0.001
Cum.Prop	0.864	0.920	0.966	0.984	0.992	0.997	1.000
Canonical R	0.548	0.165	0.150	0.094	0.063	0.049	0.037
Wilks' Lambda	0.655	0.935	0.961	0.983	0.992	0.996	0.999
Chi-Sqr	544.647	86.168	50.604	21.413	9.907	4.812	1.750
df	77.000	60.000	45.000	32.000	21.000	12.000	5.000
p-level	0.000	0.015	0.262	0.922	0.980	0.964	0.883

The species associated with scrub and ordinated at the right of the diagram, do not show any significant interspecific Mahalanobis distances, indicating a certain amount of habitat overlap. The high proportion of wrong a priori classifications and the overall low percentage correct classification (Table 5) confirm the common habitat use and the high degree of habitat overlap.

TABLE 4

Squared Mahalanobis distances (shaded) and F-values for the discrimant function between all species pairs. Significant different distances at the 0.01-level in bold, significant distances at the 0.05 level underlined.

Squared Mahalanobis Distances F-values; df = 11,1278								
	A. palustris	*L. naevia*	*P. collybita*	*P. trochilus*	*S. atricapilla*	*S. borin*	*S. communis*	*S. curruca*
A. palustris	——	1.003	**5.226**	0.961	**4.553**	2.179	1.594	0.541
L. naevia	0.735	——	**10.865**	0.557	**8.429**	**4.092**	1.276	1.075
P. collybita	**2.988**	**2.707**	——	**30.323**	1.214	**3.980**	**24.346**	**9.968**
P. trochilus	0.540	0.133	**2.369**	——	**20.348**	**6.410**	2.092	0.834
S. atricapilla	**2.692**	**2.265**	0.130	**1.987**	——	2.360	**14.884**	**7.896**
S. borin	1.528	**1.549**	**0.864**	**1.330**	0.558	——	**5.227**	**3.780**
S. communis	0.895	0.305	**1.885**	0.142	**1.443**	**1.081**	——	1.489
S. curruca	0.374	0.396	**2.062**	0.165	**1.788**	**1.271**	0.293	——

TABLE 5

A priori classification matrix of all Sylviid-warblers

Species	**Percentage correctly classified**	**A.pa**	**L.na**	**P.co**	**P.tr**	**S.at**	**S.bo**	**S.co**	**S.cu**
A. palustris	0.000	0	0	0	21	0	0	1	0
L. naevia	0.000	0	0	2	35	0	0	18	0
P. collybita	53.541	0	0	136	78	3	0	37	0
P. trochilus	69.135	0	0	13	224	2	2	83	0
S. atricapilla	1.136	0	0	77	54	2	4	39	0
S. borin	3.076	0	0	24	27	0	2	12	0
S. communis	34.441	0	0	31	181	3	2	114	0
S. curruca	0.000	0	0	5	52	0	0	12	0
Total	36.882	0	0	288	672	10	10	316	0

Territory overlap and interspecific interactions within the genera *Phylloscopus & Sylvia*

P. collybita and *P. trochilus* discriminate significantly by the amount of tall grassland and woodland within the territories (Wilks' Lambda: 0.632; approx. F (11,566)=29.915; p<0.0001: Table 6). 78.03% of the territories were well classified. 52 territories of both species overlapped in the field, while 127 were expected based on the a priori classification (χ^2=111.58, p<0.001; Table 7). This indicates that the observed territory overlap is significantly lower than can be expected based on the common habitat occupancy. Within the common habitat, *P. collybita* is the dominant species, as a result of an asymmetrical interaction in which the latter occupies more territories typical for *P. trochilus* without tolerating its presence.

All Sylvia-warblers can be discriminated based on the included vegetation variables (Wilks' Lambda: 0.738; approx. F (33,1847)=6.081; p< 0.001 (proportion of woodland and short grasslands contribute significantly: Table 8)), resulting in an overall correct classification score of 58.35% (Table 9). Especially the territories of *S. communis* (90.93% correctly classified) can be well discriminated. All territories of *S. curruca* were classified as other Sylvia-habitats, indicating a high interaction between this species and the congeneric warblers.

With the exception of the species pair *S. communis*-*S. curruca*, all territories of the congeneric pairs could be discriminated by the included vegetation variables (Table 6). Of these, only the observed coexistence of the species pair S. *borin*-*S. curruca* matched the expected coexistence

based on the a priori classification of the territories (Table 7), indicating a common habitat with territory overlap and without interspecific interactions.

Of all other species pairs (Table 7), *S. borin* was significantly dominant in potential territories of *S. atricapilla* and *S. communis*, *S. atricapilla* in potential territories of *S. curruca* and *S. communis*, while *S. curruca* was dominant only in the territories of *S. communis*. Although the latter is capable of occupying territories of the other congeneric species, it is never dominant in the interaction.

TABLE 6

Results of the discriminant analysis between congeneric Sylviid warbler pairs, with indication of the significant differentiating vegetation components.

Species-pair	Wilks' Lamda	F	p	sign. Components
P. trochilus- P. collybita	0.632	29.915	<0.001	Tall grassland, woodland
S. communis - S. curruca	0.967	1.167	0.307	
S. communis - S. borin	0.862	5.552	<0.001	woodland, *L. vulgare* shrub
S. communis - S. atricapilla	0.768	13.54	<0.001	woodland
S. borin - S. atricapilla	0.883	2.733	<0.01	*S. repens* shrub, short grassland
S. borin - S. curruca	0.7	4.756	<0.001	woodland, grey dunes
S. curruca - S. atricapilla	0.773	6.199	<0.001	short grassland, woodland

TABLE 7

Overview of the observed and expected territory overlap within congeneric Sylviid warbler pairs, with indication of the dominant species in the interaction. Total possible interactions are calculated as the sum of both species' numbers of territories; observed coexistence is derived from field data; expected coexistence is based on the discriminant analysis classification. The χ^2-coexistence is the result of frequency testing between expected and observed coexistence; χ^2-dominance is the result of frequency testing between the dominant species' and equal occupancies.

Species-pair	total possible combinations	obs. coexistence	exp. coexistence	χ^2-coexistence	p	dominant species in interaction	χ^2-dominance	p
P. trochilus- P. collybita	578	52	127	111.58	< 0.001	*P. collybita*	124.74	< 0.001
S. communis - S. curruca	400	47	69	4.89	0.027	*S. curruca*	138.00	< 0.001
S. communis - S. borin	396	41	74	11.08	< 0.001	*S. borin*	18.27	0.011
S. communis - S. atricapilla	507	42	129	28.34	< 0.001	*S. atricapilla*	87.21	< 0.001
S. borin - S. atricapilla	205	18	64	26.41	< 0.001	*S. borin*	78.13	< 0.001
S. borin - S. curruca	134	22	31	1.91	> 0.05			
S. curruca - S. atricapilla	245	18	63	29.95	< 0.001	*S. atricapilla*	26.71	< 0.001

TABLE 8

Summary of the discriminant analysis of the Sylvia-warblers, based on the vegetation composition within the species' territories (Wilks' Lambda= 0.738; F_{approx} (33,1847) = 6.0812, p<0.001).

Vegetation type	Wilks' Lambda	Partial Lambda	p-level	R^2
Grey dunes	0.743	0.993	0.207	0.051
L. vulgare shrub	0.745	0.991	0.138	0.016
Marram dunes	0.742	0.995	0.364	0.131
Mixed shrub	0.744	0.992	0.174	0.528
S. nigra-shrub	0.739	0.998	0.791	0.283
S. repens-shrub	0.740	0.998	0.713	0.058
Sandy dune slack	0.740	0.997	0.614	0.066
Short grassland	0.746	0.989	0.078	0.371
Tall grassland	0.741	0.995	0.409	0.080
Woodland	0.864	0.854	0.000	0.423
Young dune slack	0.741	0.996	0.510	0.059

TABLE 9

A priori classification matrix of the Sylvia-warblers

Species	% correctly classified	S.at	S.bo	S.co	S.cu
S. atricapilla	40.340	71	4	101	0
S. borin	3.0763	20	2	43	0
S. communis	90.936	28	2	301	0
S. curruca	0.000	4	0	65	0
Total	58.346	123	8	510	0

DISCUSSION

Habitat selection

CRAMP (1992) gives a general habitat description of the discussed species, based on numerous studies in Britain, Continental Europe and Northern Africa. The Chiffchaff *P. collybita* is basically a bird of mature lowland woodland without a dense canopy and with a fairly copious variety of medium or tallish undergrowth. *P. trochilus* prefers scrub, second growth and transitions to more open woodland. An analogous habitat segregation between both species was observed by FULLER et al. (1989) in a mixed coppiced woodland in Kent, England. SAETHER (1983) studied habitat selection and foraging niches in an area of sympatry in Norway. There the species were characterised by a considerable overlap in their foraging niches but were found to occupy almost mutually exclusive territories. Especially the Willow warbler selected a greater variety of habitats (but was mainly found in the early stages of woodland succession) than did the Chiffchaff.

Of the Sylvia species, *S. curruca* flourishes in habitats intermediate between extensive closed forest and open country, restoring to well-spaced often tall bushes, scrubs, taller than those preferred by the Whitethroat *S. communis.* The latter's basic requirement is a patchy, low, fairly dense cover, natural or planted. In contrast to the former species, S. *borin* and *S. atricapilla* are primarily woodland species, but in comparison to *S. atricapilla*, *S. borin* prefers a more even open canopy accompanied by much fairly dense and tall scrub or a shrub layer. FULLER et al., 1989 confirm these results in their study from Kent, England. With the exception of *A. palustris*, the other Sylviine species are characteristic for low, herbaceous vegetation. As a result of his findings, CODY (1978) states that vegetation above 1.5 m. in height supports both *Phylloscopus* and *Sylvia* representatives, below 1.5 m in height just *Sylvia* but with *Acrocephalus* and *Locustella* in combination with the short vegetation patches. In the Flemish coastal dunes, however, *Phylloscopus* may be present in lower *Salix repens* or *Hippophae rhamnoides* shrubs, where it shows a habitat preference analogous to that of *L. naevia*. The latter, however, has a somewhat larger territory size (CRAMP & BROOKS, 1992; BONTE, personal data).

Our results confirm these general findings. According to the discriminant function analysis of the species' territories, Sylviine Warblers in the Flemish coastal dunes have distinctive habitat preferences, based on the territory-specific vegetation composition. The plot with confidence intervals characterises *P. collybita* and *S. atricapilla* as two species of tall dune woodlands and higher scrubs. The other Sylviine species are more or less typical for scrubland. Only *S. borin* is positioned between dune woodlands and scrubs, confirming Cramp's classification. *A. palustris* and *S. curruca* are typical for homogeneous mixed scrub, while especially *S. communis* is typical for scrub-grassland mosaics. The presence of low vegetation in the species' territory is, however, of minor importance for the overall ordination (low eigenvalue) and probably only important for the settlement of *S. communis*.

Although the discriminant-plot does not show any habitat overlap between congeneric species, mahalanobis distances and a priori classifications indicate a certain amount of habitat overlap between *S. curruca* and *S. communis*, and a low but significant distance between *S. borin* and *S. atricapilla*. For the other species pairs, mahalanobis distances indicate a substantial habitat overlap between the typical scrub species. The habitat segregation between *S. borin* and *A. palustris* is also non-significant, indicating a similar habitat preference.

CODY (1978) showed in English habitats (Yorkshire) that the species pairs *Phylloscopus trochilus-P. collybita* (intermediate scrubland), *Sylvia atricapilla-S. borin* (tall woodland) and *S. communis-S.curruca* (low shrub) considerably overlap in preferred habitat type. Only a minor segregation was present between *S. atricapilla* and *S. borin* in higher vegetation, where *S. atricapilla* was more abundant in habitats with both dense canopy and dense middle layers and *S. borin* was alone present in habitats with dense but lower canopy that inhibits a bush layer but encourages thick ground cover lower than 1 m. Similarly as in our study area, *S. communis* shows an extensive habitat overlap with *S. curruca* in scrub with taller emergent shrubbery. In England, *S. communis* overlaps in more open bushy habitats with *S. undata*; a species which was absent in our study area.

In the Flemish coastal dunes, only the habitat segregation between the two *Phylloscopus* species is very clear, probably because of the high proportion of woodland in our study area, whereas CODY only studied habitat segregation in scrubland, with only a minor proportion of woody vegetation.

Despite these significant habitat differences, shrub- and canopy-inhabiting warblers have a broad habitat choice, which accounts for the low success of habitat occupancy predictions, based on discriminant analysis with the vegetation characteristics. CODY (1978) did use vegetation height measures instead of vegetation typology. These height-classes are also represented in our distinguished vegetation types ordered from the tallest to the shortest vegetation: woodland, mixed scrubs, *Sambucus nigra* scrubs, *Hippophae*-scrubs, *Ligustrum* scrubs, *Salix repens* scrubs, high dense grasslands, short grasslands and grey dunes and blond dunes.

The a priori classification of *Phylloscopus trochilus, P. collybita* and *P. sibilatrix* in Sweden (CODY, 1978) resulted in a correctness of 67 %. In the Flemish coastal dunes, this classification is better, probably because of the absence of *P. sibilatrix*. In the same study, interspecific classifications were a priori obtained for our discussed *Sylvia* species supplemented with *S. undata* in Britain and supplemented with *S. nisoria* in Sweden. In both cases,

the correct rate of classification was 54%, slightly lower than in our study (58.3 %). In both studies of CODY (1978), especially *S. curruca-S. communis* and *S. atricapilla-S. borin* displayed considerable habitat overlap, a similar result to that obtained by MASON (1976).

Interspecific territoriality

Although habitats of the congeneric Sylviine Warblers overlap, only a minor proportion of territories overlap, probably as a result of interspecific territoriality. In this contribution, only interspecific territoriality was studied between congenerics, because of their similar morphology (same body and wing size, same weight), territory size and feeding preference.

In the Flemish coastal dunes, territory overlap occurs between all *Phylloscopus* and *Sylvia* species pairs. Only in the case of the species pair *S. borin* and *S. curruca* does the expected coexistence (territory overlap) match the observed coexistence based on the a priori classifications. In all other species pairs, one species is dominant in the other's potential territory. Apparently, *S. borin* is a strong competitor since it coexists with *S. curruca* and dominates *S. atricapilla* and *S. communis* in their potential habitat. *S. atricapilla* is dominant over *S. curruca* and *S. communis*. The latter will never dominate other congenerics. *P. collybita* is dominant when *P. trochilus* habitats are occupied.

Our results confirm and supplement the data of CODY (1978; 1985) who also studied interspecific territoriality based on observed and expected common habitat use in scrubland of the Beyershamn reserve (Öland, Sweden). He also found that *S. borin* excluded *S. communis* and *S. nissoria*, but not *S. curruca*. In Krapperup Wood (Hörby, mainland, Sweden), however, *S. borin* excluded *S. curruca* weakly, with the existence of territory overlap. This interaction was, however, variable and certainly far weaker than could be expected on the basis of other species pairs (CODY, 1978). The other possible interactions *curruca - atricapilla* and all *S. communis* combinations could not be studied because of their rarity. In this study site, *S. borin* excluded *S. atricapilla* via song competition. This contrasted to the Yorkshire site, where both species occupied territories in a symmetrical way, as if they were members of the same species.

The time of arrival in the breeding areas does not guarantee dominance of the first arrival since *S. borin* is the last *Sylvia*-species returning from the winter quarters. With the exception of the species pair *S. borin-atricapilla*, congeneric species typical for higher scrub-types dominate sister species of lower scrubland. The dominance of *S. borin* over the other congenerics explains its potential settlement in both woodlands and scrubs. Besides song competition (song convergence: when a song of a neighbouring species is incorporated into the repertoire and the neighbour is thereby discouraged from interspecific territory overlap) as investigated by CODY (1987), direct aggression may be the basis of the interspecific territoriality. Both Cody's and our results contradict this hypothesis since BERTHOLD (1978) reported a higher amount of direct agression in *S. atricapilla* over *S. borin*. However, the observed territory interactions in the species pairs involving *S. borin, S. curruca* and S. *communis* can be explained by direct aggressiveness (discussed per species in CRAMP & BROOKS, 1992), especially in the case of *S. curruca* and *S. communis*, which have broadly overlapping habitat preferences. CODY (1978) observed effective aggressive interactions between both species, resulting in non-overlapping, often contiguous territories. Direct aggression and song convergence between *S. borin* and *S. curruca* (which has an aberrant song in comparison to the other Sylvia-members) has never been observed and possibly explains the species' coexistence.

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Developmental instability in relation to stress and fitness in birds and moths studied by the Laboratory of Animal Ecology of the University of Antwerp

Stefan Van Dongen [1], Luc Lens [1,2] and Eric Matthysen [1]

[1] **Department of Biology, University of Antwerp, Universiteitsplein 1, B-2610 Wilrijk, Belgium**
[2] **Department of Ornithology, National Museums of Kenya, PO Box 40658, Nairobi, Kenya**

ABSTRACT. Evolutionary and conservation biologists are in need of simple surrogate measures of fitness and the action of stress on fitness components. Fluctuating asymmetry, an estimate of developmental instability, has been suggested to reliably reflect stress and fitness but evidence is highly heterogeneous. This heterogeneity is confirmed by the results we obtained from three different projects at the Laboratory of Animal Ecology that we review in this paper. In seven bird species inhabiting three indigenous cloud forests in Kenya, asymmetry closely related to stress and/or fitness, yet the underlying mechanisms appeared complex. On the other hand, in two moth species the link between asymmetry and both stress and fitness was less clear or even absent. These results call for more large-scale studies in order to identify factors that allow predicting if and when asymmetry reflects stress and/or fitness.

KEY WORDS: developmental instability, bilateral asymmetry, stress, fitness, review.

INTRODUCTION

Estimating fitness

Individual fitness is the result of various processes, many of which are difficult to establish directly, especially in free living organisms. Nevertheless, individual fitness takes a central place in many areas of research such as evolutionary and conservation biology. The availability of a universal measure of fitness that can be obtained easily and rapidly and that is ubiquitous across taxa and locations will facilitate many aspects of evolutionary research and the development of conservation plans. A measure that has been claimed to have these properties is fluctuating asymmetry, as an estimator of developmental instability (see below). However, a number of recent overviews of the available literature have indicated otherwise, pointing out that there are large inconsistencies in the literature (e.g. LEUNG & FORBES, 1996a; CLARKE, 1998a; BJORKSTEN et al., 2000). At present it is not clear which factors influence the FA-fitness and FA-stress association at either the population or individual level, and this calls for further research (e.g. VAN DONGEN & LENS, 2000b).

We recently argued that there is an urgent need for comparative studies attempting to identify factors that influence the FA-stress association (VAN DONGEN & LENS, 2000b). However, given the sensitivity of FA-studies to poor statistical analyses, low statistical power due to small sample sizes and statistical properties of FA, publication bias (SIMMONS et al., 1999; PALMER, 1999), and selective reporting (CLARKE, 1998a), objective and unbiased meta-analyses are difficult to perform. In this paper we present an overview of the results of three projects that were initiated at the Laboratory of Animal Ecology (Antwerp) and discuss directions for future research.

Fluctuating asymmetry as estimator of fitness

Fluctuating asymmetry (FA) is the most commonly used measure of developmental instability (DI), and can be defined as small directionally random deviations from perfect symmetry of otherwise symmetrical bilateral traits (LUDWIG, 1932; PALMER & STROBECK, 1986; PALMER,

Corresponding author: S. Van Dongen,
e-mail: stefan.vandongen@uia.ua.ac.be

1996). DI, the set of processes that are assumed to be estimated by FA, reflects the cumulative effects of mistakes during ontogeny and individual specific mechanisms that counteract these faults (PALMER & STROBECK, 1992). The underlying assumptions are that the development of any trait is disturbed by random noise, that these mistakes accumulate over time and that in the course of evolution mechanisms have evolved that attempt to correct for these mistakes. The first two processes are termed developmental noise, the latter developmental stability (DS). Thus, noise causes traits to deviate from their expected growth trajectory (as predicted by genotype and environment) whereas DS is assumed to counteract the effects of noise. DI indicates the combined effect of both noise and stability, since it is impossible to disentangle both purely from asymmetry values. When expressed in statistical terms, any trait value can be viewed as a sample from a normal distribution with mean equal to the expected value and variance being the joint and opposite action of noise and stability (LEUNG & FORBES, 1996b).

Since the expected trait value, given an individual's genotype and the environment in which it developed, is seldom known, it is impossible to estimate the variance of that distribution from an observed trait value (because a mean and variance cannot be estimated simultaneously from a single observation). This problem can be circumvented by measuring traits that have the same expected value, such as bilaterally symmetrical traits. The difference between left and right can be expected to follow a normal distribution with zero mean and variance equal to twice the developmental instability (WHITLOCK, 1996).

One could postulate an association between DI (and thus FA) and fitness if the mechanisms underlying developmental stability are energetically costly. When conditions (either genetic or environmental or both) during development are optimal, individuals can be expected to be able to allocate sufficient energy into processes of developmental stability and have high expected fitness. Under unfavourable situations, less energy might be available to buffer development and an increased DI may then coincide with fitness loss. Under this hypothetical model, the association between fitness and DI can be expected to critically depend on species-, trait- or even individual-specific strategies of allocation.

Specific hypotheses

Because at present there are no specific guidelines that predict when FA can be reliably used as a predictor for fitness, for each application a number of hypotheses need to be tested:

Does FA increase with stress and is this response trait specific? For population-level analyses, the most important hypothesis to test is whether FA increases with stress (either environmental or genetic). Secondly, it should be tested if responses are trait-specific such that it depends on the chosen trait whether an association is found. In other words, one should investigate if variation in population-level FA correlates among traits [i.e. is there evidence for a so-called population asymmetry parameter (further called PAP, CLARKE, 1998b)]. Trait-specific response, or even the absences of any association, clearly hampers the general use of FA as estimator of stress and fitness.

Are the effects of environmental and genetic stress additive? It has been hypothesised earlier that FA might only be a sensitive estimator of environmental stress in the presence of genetic stress (inbreeding, mutation or disruption of genomic co-adaptation) (PARSONS, 1992). Therefore, an interaction between the effects of environmental and genetic stress should be tested.

Does FA reflect individual fitness? At the individual level, FA and DI have been argued to reflect fitness and/or (genetic) quality. In order to reflect genetic quality, FA and DI should have a genetic basis (see hypothesis 4) and reflect at least some component of fitness. The association between FA and other factors, however, underestimates associations between these factors and DI. Since FA estimates a variance with two datapoints (i.e. left and right trait value) it is only a very crude estimator of DI (PALMER, 1994; WHITLOCK, 1996; HOULE, 1997). This results in a downward bias of observed associations, which can be corrected for using the concept of 'hypothetical repeatability' as developed by Whitlock (1996, 1998).

Is FA (and DI) genetically heritable? A genetic background is required to expect any association between individual genetic quality and FA, whereas it would complicate the use of FA at the population level since this would imply that populations could evolve and adapt to the changing environmental conditions. Therefore, although only poorly understood, the genetic background and evolutionary potential of FA and DI are crucial for interpreting observed patterns (MØLLER & THORNHILL, 1997 vs. e.g. MARKOV & CLARKE, 1997; VAN DONGEN & LENS, 2000a; VAN DONGEN, 2000b).

Which type of asymmetry occurs and how do they relate to stress? Next to FA, two other forms of asymmetry may occur. With directional asymmetry (DA) the mean value of the asymmetry differs from zero, while for antisymmetry (AS) the distribution is bimodal. It has often been argued that DA and AS have a genetic basis and do not reflect the cumulative result of developmental errors (e.g. PALMER & STROBECK, 1992). Classical examples of this are different species of fiddler crabs where either DA or AS may occur when one claw grows much larger than the other does. On the other hand, both theoretical and empirical results show an association between either DA or AS and environmental and genetic perturbations accumulated over the past decade (e.g. MCKENZIE & CLARKE, 1988; GRAHAM et al., 1993a; 1993b; LEAMY et al., 1999). At present it is not clear if and when DA and/or AS increase with perturbations and if they reflect DI. Nevertheless it is crucial to distinguish between these different forms of asymmetry in order to perform correct statistical analyses.

MATERIAL AND METHODS

Three projects

Taita Hills project: Bilateral trait asymmetry was studied in one to three traits of seven forest bird species (listed in Table 1) in the Taita Hills of southeast. In addition, a subset of 237 *Turdus helleri* was genotyped with six polymorphic microsatellite-DNA markers, and individual inbreeding coefficients were calculated following Ritland (1996) (details in GALBUSERA et al., 2000; LENS et al., 2000). Indigenous cloud forest currently covers less than 400 ha in 12 forest patches, eight of which are smaller than 5 ha, in Kenya (map and details in BROOKS et al., 1998; GALBUSERA et al., 2000). Levels of FA and inbreeding were studied in bird populations inhabiting the three largest of the Taita forest fragments. Based on congruency in the patterns of habitat disturbance (WILDER et al., 2000) and avian survival rates (LENS et al., 2000), levels of 'environmental stress' were considered highest in fragment CH (50 ha), intermediate in fragment NG (90 ha), and lowest in fragment MB (200 ha).

Winter moth project: The study of the population structure of the winter moth (*Operophtera brumata* L.) in relation to habitat fragmentation and local adaptation to individual host trees was initiated in 1991. During the course of this research project, analyses of population- and individual-level fitness in relation to tibia asymmetry, were performed at various stages. We investigated the effect of habitat fragmentation and individual heterozygosity at five allozyme loci in one large area (>200 ha) and two small fragments (<2ha) near Antwerp (VAN DONGEN, 1997), estimated the heritability of FA and DI in a full-sib breeding experiment (VAN DONGEN et al., 1999d) and investigated the association between individual FA and several fitness components in a series of experiments (VAN DONGEN et al., 1999d; 1999e). We refer the reader to the individual studies for more details.

Indian meal moth project: Effects of environmental stress on FA were studied in a laboratory culture of the Indian meal moth (*Plodia interpunctella*). Using a full sib breeding design, offspring from a total of 30 couples were distributed over a total of nine different combinations of two treatments (i.e. three different food qualities and three different densities). Front leg tibia asymmetry was measured for the surviving offspring, together with an independent measure of fitness (i.e. body length). In this way, effects of presumed environmental stress, of genotype and possible genotype¥environment interactions could be tested for both FA and two independent measures of fitness (body size and survival probability).

Statistical analyses

Although we refer to the particular case studies for details on the performed analyses, in each case three steps were carefully carried out. Lens and Van Dongen (2001) and Van Dongen et al. (2001a) give an overview of the different methods.

Firstly, we obtained unbiased estimates of FA at the individual and/or population level. This was achieved by a mixed regression approach (VAN DONGEN et al., 1999a; VAN DONGEN, 2000a). These estimates were then compared across populations (by likelihood ratio test or Levene's test) or individual estimates were used in subsequent analyses. Secondly, between-trait correlations in FA and heritabilities of FA were transformed into (upper bounds of) patterns in DI following Whitlock (1996, 1998) and Van Dongen (1998). Finally, blends of different forms of asymmetry were distinguished by mixture analysis (VAN DONGEN et al., 1999b).

RESULTS AND DISCUSSION

Table 1 summarises tests of the above listed hypotheses. Associations between FA and both stress and fitness appear very heterogeneous. Roughly, in the Taita Hills project most hypotheses were confirmed and FA appeared to reflect stress and fitness at both the individual and population level, regardless of the trait studied. Most importantly, the different traits studied developed at different stages during the life of these birds emphasising that the FA-fitness associations hold throughout large parts of their lifespan. The results also suggest that the underlying mechanisms are relatively complex. For *Turdus helleri* environmental stress and inbreeding interacted with each other such that more inbred individuals showed an increase in asymmetry in the most disturbed area only. This association was weaker but still statistically significant in the intermediately disturbed area and absent in the relatively undisturbed area (LENS et al., 2000). Likewise, the association between individual asymmetry and fitness was influenced by the degree of habitat disturbance. In the most disturbed area a relatively strong negative association between individual FA and survival was found, while no association occurred in the two other areas. In addition, directional asymmetry appeared to occur more frequently in more disturbed areas for at least four species (Table 1; LENS & VAN DONGEN, 2000). Although such shifts in types of asymmetry have been observed in other species (see above), its relationship with DI is not entirely clear (but see GRAHAM et al., 1993a).

In the two moth studies, associations between asymmetry and both stress and fitness are less obvious or even absent (Table 1). The only association between FA and fitness was detected in the winter moth, where females were more likely to re-mate when the first mating occurred with an asymmetric male. The more asymmetric males are thus likely to loose paternity. However, this association was observed in a laboratory experiment, and re-mating is considered very unlikely in the field (VAN DONGEN et al., 1999e). The absence of an FA-fitness association could not be attributed to small sample sizes or inappropriate statistical analyses. Furthermore, significant heritability

TABLE 1

Overview of results obtained at the Laboratory of Animal Ecology of the University of Antwerp, testing eight specific hypotheses. An * indicates that the hypothesis is confirmed, NS that it is not supported by the data. Specific hypotheses are: (i) does environmental stress increase FA and thus DI; (ii) are different traits affected in a similar way [i.e. is there evidence for a Population Asymmetry Parameter (PAP)]; (iii) do FA and DI increase with inbreeding or decrease with heterozygosity; (iv) is there evidence for an interaction between environmental and genetic stress; (v) are FA and DI heritable; (vi) is individual fitness related to FA and DI; (vii) is FA correlated among different traits [i.e. is there evidence for an individual asymmetry parameter (IAP)]; (viii) Do other forms of asymmetry occur more frequently with increasing stress.

Species:	(i)	(ii)	(iii)	(iv)	(v)	(vi)	(vii)	(viii)
Taita hills project:								
Turdus helleri (Gmelin) [1]	*	*	NS	*	-	*	*	-[6]
Nectarinia olivacea (Smith)[1]	*	*	-	-	-	-	*	*
Andropadus milanjensis (Shelley)[1]	*	*	-	-	-	-	*	-[6]
Zosterops silvanus (Heuglin)[1]	*	*	-	-	-	-	*	*
Pogonocichla stellata (Vieillot)[1]	*	*	-	-	-	-	*	*
Phylloscopus ruficapillus (Sundevall)[1]	*	-	-	-	-	-	-	-
Phyllastrephus cabanisi (Sharpe)[1]	*	-	-	-	-	-	-	*
Winter moth project:								
Operophtera brumata (L.)[2]	*	(*)[4]	NS	NS	*	(*)[5]	(*)[4]	NS
Indian meal moth project:								
Plodia interpunctella (Hubner)[3]	NS	-	-	-	NS	NS	(*)[4]	NS

[1] Details in Lens et al. (1999; 2000); Lens & Van Dongen (1999; 2000) and unpublished results
[2] Details in Van Dongen (1997) and Van Dongen et al. (1999c; 1999d; 1999e)
[3] Details in Van Dongen et al. (2001b)
[4] Correlations between traits are likely to be confounded with common developmental processes (Van Dongen et al., 1999c)
[5] Although significant under laboratory conditions, in the field it is considered unlikely to occur (Van Dongen et al., 1999e).
[6] Insufficient data from the most disturbed area were available in this analysis (Lens & Van Dongen, 1999)

of FA, as observed for the winter moth, could not explain the weaker associations, since FA was not heritable in the Indian meal moth in which no indication of any FA-fitness association was found. One possible explanation could be that we did not measure the appropriate trait. We measured the tibias of the three pairs of legs, but these may not be considered as independent growth events (Van Dongen et al., 1999c). Since in *Turdus helleri* an association between FA and fitness was only found in the most disturbed area, one could argue that the environmental stress was not sufficient in the two moth species for any effect to be detectable. Nevertheless, for both species the most severe treatment led to at least 50% mortality (Van Dongen et al., 1999d; 1999e; 2001b).

In summary, studies performed by us show that at least in some cases FA may provide a reliable surrogate measure of fitness and stress, but also that associations may be less straightforward or even completely absent. However, even in situations where FA-stress association 'works, the underlying mechanism relating to this association appears relatively complex through an interaction between environmental and genetic stress, a differential expression of FA-fitness associations with habitat disturbance, and the occurrence of other forms of asymmetry. Developing guidelines that predict if an when FA is expected to relate to stress requires that the underlying developmental mechanisms and related biochemical pathways that lead to asymmetry are unravelled first.

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The interstitial Rotifera of a tropical freshwater peat swamp on Phuket Island, Thailand

Hendrik Segers[1] **and Supenya Chittapun**[2]

[1]**Department of Biology, Ghent University**
K. L. Ledeganckstraat 35, B – 9000 Gent (Belgium)
[2]**Dept. Biology, Faculty of Science, Prince of Songkla University**
Hat Yai 90112, Thailand

ABSTRACT. We studied the Rotifera inhabiting the psammon of a coastal freshwater peat swamp on Phuket Island, Thailand, in order to provide a first report on interstitial rotifers from tropical regions. The records total 19 species, three of which we describe as new to science. Four species are regional endemics, one is Oriental and two are widespread but very rare. Six taxa are new to Thailand. The results indicate a high endemicity rate, however, the scarcity of information on the distribution of tropical interstitial rotifers, illustrated by the description of one of the new species from Thailand and Bolivia, precludes generalisations at this time.

KEY WORDS: Rotifera, interstitial fauna, zoogeography, taxonomy, Thailand.

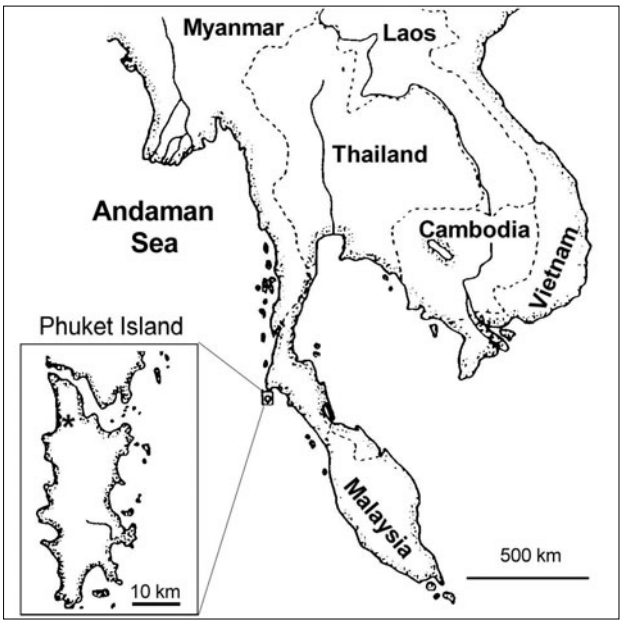
INTRODUCTION

When compared to plankton and even littoral Rotifera, the communities of rotifers inhabiting interstitial environments are rarely studied. The peculiar taxa occurring in the psammon are rare, and/or insufficiently known. Many are presently considered endemic, but it is as yet unclear to what extent this is factual or an artefact of insufficient sampling. Some studies exist on interstitial freshwater and marine European and Northeast American habitats (e.g. WISZNIEWSKI, 1934; REMANE, 1949; PENNAK, 1951; ALTHAUS, 1957a, b; TZSCHASCHEL, 1979, 1980, 1983; TURNER, 1990; SEGERS, 1998; RADWAN & BIELANSKA-GRAJNER, 2001), but hardly any information is available on interstitial rotifers from tropical regions.

In order to contribute to the knowledge of tropical interstitial rotifer communities, we sampled the hygropsammon (see WISZNIEWSKI, 1934) of a coastal freshwater habitat on Phuket Island, Southern Thailand, Mai-Khao peat swamp. The plankton rotifer fauna of this swamp has already been studied by CHITTAPUN et al. (1999).

MATERIAL AND METHODS

Mai-Khao peat swamp is a pristine coastal peat swamp on Phuket Island, Southern Thailand (Map). The swamp water is slightly acidic (pH 5.6-5.9), and brown-coloured



Map. – Situation of the Mai-Khao peat swamp (*) on Phuket Island

Corresponding author: H. Segers, e-mail: Hendrik.Segers@rug.ac.be

(turbidity 4-18 NTU). The swamp is freshwater, but some influence of the Andaman Sea, from which the swamp is separated by a dike, cannot be excluded. The conductivity is 1.98-2.90 mS.cm^{-1} (CHITTAPUN et al., 1999).

Two samples of hygropsammon were taken on 28 July 1999, by collecting c. 50 cc of sand from the top 0.5 cm of hygropsammon. Fixation was done with formaldehyde (4%). Rotifers were isolated by searching the samples using a Wild M10 dissection microscope, and examined and drawn using an Olympus CH2 microscope with drawing tube. Scanning electron microscopy (SEM) was performed using a JEOL JSM-840 microscope on trophi material processed following SEGERS (1993) and SEGERS & DUMONT (1993). Permanent slides of types are deposited in the collections of the royal Belgian Institute for Natural Sciences, Brussels, Belgium (KBIN), of the department of Biology of Ghent University, Ghent, Belgium (RUG), of Prince of Songkla University, Hat Yai, Thailand (PSU) and of the University of Antwerp, R.U.C.A. campus, Antwerp, Belgium (RUCA). All measurements are in µm.

TAXONOMY

Of the 19 rotifer species found (Table 1), three are new to science, and the trophi morphology of two more species is insufficiently known.

TABLE 1

List of Rotifera in the psammon of Mai-Khao peat swamp

*New to Thailand, †Endemic to Thailand, [1]Oriental endemic
1: single specimen; RR: 1-5 specimens, R: 5-10 specimens, C: >10 specimens.

Brachionus urceolaris (Müller, 1773): 1
Cephalodella innesi Myers, 1924: RR
**C. megalocephala* (Glascott, 1893): R
**C. plicata* Myers, 1924: C
Colurella colurus (Ehrenberg, 1830) f. *compressa* Lucks, 1912: RR
,†Colurella psammophila*** **new species: R**
Colurella obtusa (Gosse, 1886): R
†C. sanoamuangae Chittapun, Pholpunthin & Segers, 1997: C
**Encentrum longidens* Donner, 1943: R
,†Encentrum pornsilpi*** **new species: C**
[1]Lecane acanthinula (Hauer, 1938): RR
L. bulla (Gosse, 1851): C
L. hamata (Stokes, 1896): 1
L. obtusa (Murray, 1913): C
L. pyriformis (Daday, 1905): 1
L. rhytida Harring & Myers, 1926: C
†L. segersi Sanoamuang, 1996: M: C
Lepadella desmeti* **new species: R**
Limnias melicerta Weisze, 1848: 1
Trichocerca tenuior (Gosse, 1886): R

Cephalodella plicata Myers, 1924
(Figs 1-2)

MYERS in HARRING & MYERS (1924) p. 483 figs 28: 3-4; KOCH-ALTHAUS 1963 p. 403 fig. 12a, b; NOGRADY et al. (1995) p. 119-120 fig. 163.

Comment

There are quite a few records of this species from Europe and North America, and one from New Zealand (DE RIDDER & SEGERS, 1997). Of its trophi, of which the original description (reproduced in NOGRADY et al., 1995) lacks

Figs 1-2. – *Cephalodella plicata* Myers, 1924, SEM photographs of trophi. 1: ventral, 2: id., detail. Scale bars: 1µm.

detail, there exists only a single, rather poor illustration (KOCH-ALTHAUS, 1963). We therefore present SEM pictures of this structure (Figs 1, 2). The trophi concur to WULFERT'S (1938) type A, with rounded rectangular basal apophysis without teeth on the inner margins; symmetrical rami with small alulae; rod-shaped manubria with a minute pore approximately medially; and broad fulcrum. Especially the latter feature is noteworthy, as it occurs in only two other species of *Cephalodella* (SEGERS & PHOLPUNTHIN, 1997).

Colurella psammophila new species
(Figs 3-5)

Material: Holotype (RIR 115) and one paratype (RIR 116) in KBIN (IG 2925); three paratypes on one slide in RUG; one paratype in PSU. All collected in the hygropsammon of the type locality, Mai-Khao peat swamp, Phuket Island, Thailand, on 28 July 1999.

Differential diagnosis

Colurella psammophila n. sp. resembles *C. colurus* f. *compressa* (Fig. 6), but has a relatively higher lorica (length: height 1.73-1.85 in *C. psammophila* n. sp. versus 1.9-1.98 in *C. colurus* f. *compressa*), and a shallower ventral sulcus. The outline of its head aperture margin is not an evenly curved outline as in *C. colurus* f. *compressa*, but has a more or less straight dorsal, and ventral part. *C. psammophila* n. sp. could also be mistaken for *C. obtusa,* but this species is smaller, and has shorter toes.

Description

Parthenogenetic female (male unknown): lorica laterally compressed, ventral sulcus shallow. Head aperture margins dorsally and ventrally straight, medially curved. Dorsal margin anteriorly straight, evenly curved from medially onwards. Minute openings to lateral antennas present postero-laterally. Head aperture with deep ventral and dorsal sinuses, dorsal foot aperture without dorsal notch, no lorica extensions lateral to the foot. Foot with three pseudosegments, the distal one approximately 1.5 times as long as the two proximal ones. A sensorial organ present mid-dorsally on the distal foot pseudosegment. Toes equal, straight to weakly curved.

Measurements: lorica length 65-81, height 35-47, width 23. Second foot pseudosegment 5.2-5.7, third foot pseudosegment 6.8-8.9, toe length 30-38.

Distribution and ecology

Colurella psammophila n. sp. is only known from the hygropsammon of the type locality, a pristine freshwater coastal peat swamp on Phuket Island, Thailand. The animal was not found during previous studies of the plankton of the swamp, so it is assumed interstitial. It co-occurred with, amongst others, *C. colurus* f. *compressa* and *C. obtusa*.

Etymology: The name of the new species is an adjective, referring to the species' habitat.

Encentrum (Encentrum) pornsilpi new species
(Figs 9-11, 17-20)

Material: Holotype (RIR 117) and one paratype (RIR 118) in KBIN (IG 29152); one paratype in RUG, one in PSU, one in RUCA. All collected in the hygropsammon of the type locality, Mai-Khao peat swamp, Phuket Island, Thailand, on 28 July 1999.

Differential diagnosis

Encentrum pornsilpi n. sp. is a close relative of *E. marinum* (Dujardin, 1841) and *E. spatiatum* Wulfert, 1936. It differs from *E. marinum* by its slender trophi, trunk without lateral sulci and non-stalked gastric glands, and from *E. spatiatum* by its rounded gastric glands, and absence of ventral body in the trunk.

Description

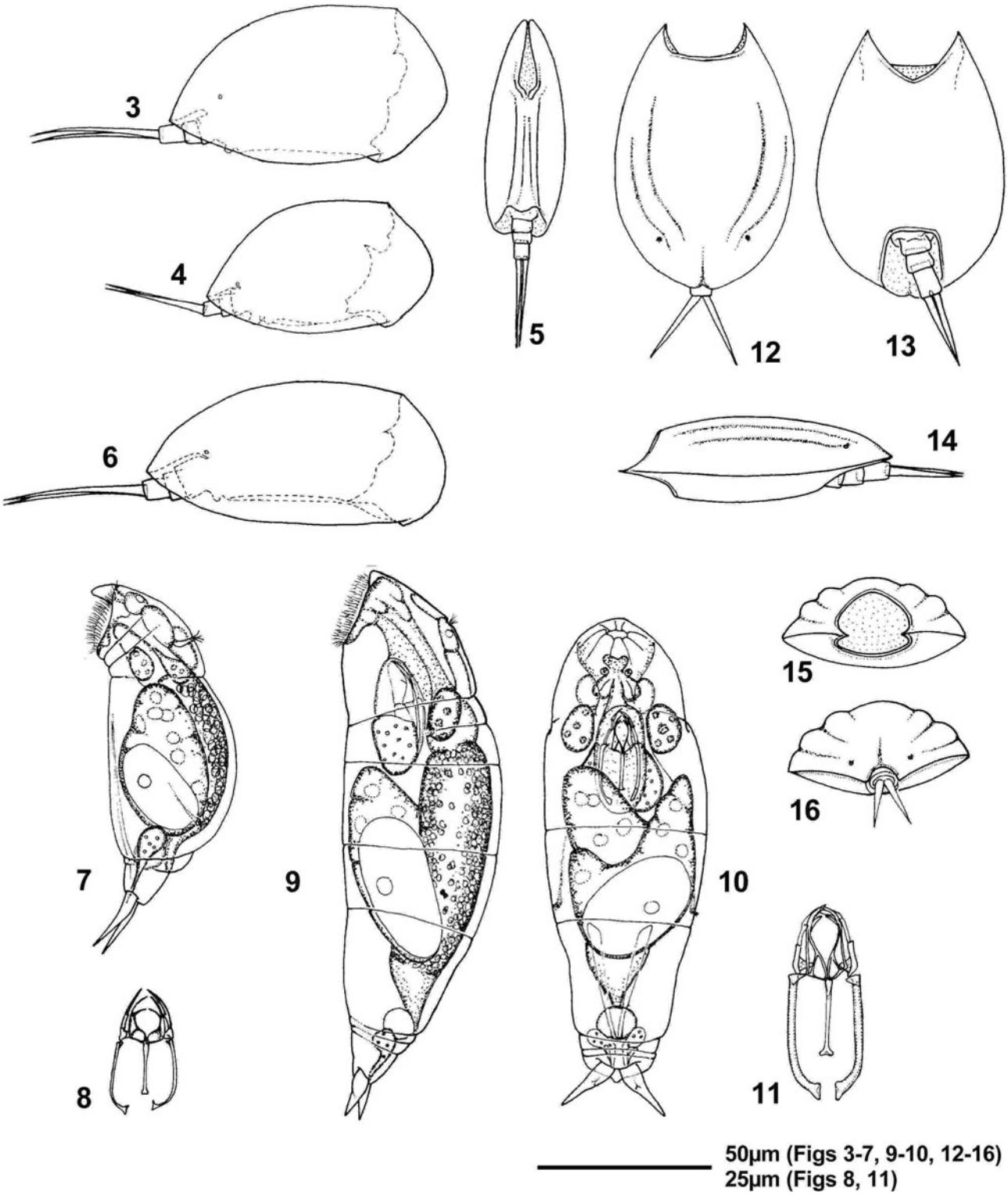
Parthenogenetic female (male unknown): Body elongate, fusiform; cuticle soft, transparent. Head c. 1/3 total length. Rostrum small, short and rounded. Corona slightly oblique, no palps observed. Trunk with weak constrictions. Tail absent. Foot short, conical in lateral view. Toes short, c. 1/8-1/10 total length, bases swollen, slightly decurved ventrally, clearly separated and with papilla between toes. No eyespots, but with two light-refracting globules in the subcerebral glands. Salivary glands terminal. Proventriculus present. Gastric glands large, ovate. Pedal glands clubbed, foot-length.

Trophi small, elongate, slender. Rami longer than wide, outer margin of rami slightly concave laterally, angular posteriorly. Each ramus terminally with single, incurved apical tooth, anterior to this tooth a preuncinal tooth set at right angle to axis; this tooth with a minute medial knob whereupon the ventral uncinal apophysis rests. Fulcrum as long as, or longer than the rami, posterior end with indented basal plate. Unci single-toothed, curved, long and slender. Tooth shaft length small, dorsal and ventral apophyses present. Intramallei long, elongate-triangular in ventral view. Manubria shorter than incus, a triangular expansion proximally, distally strongly incurved and knobbed.

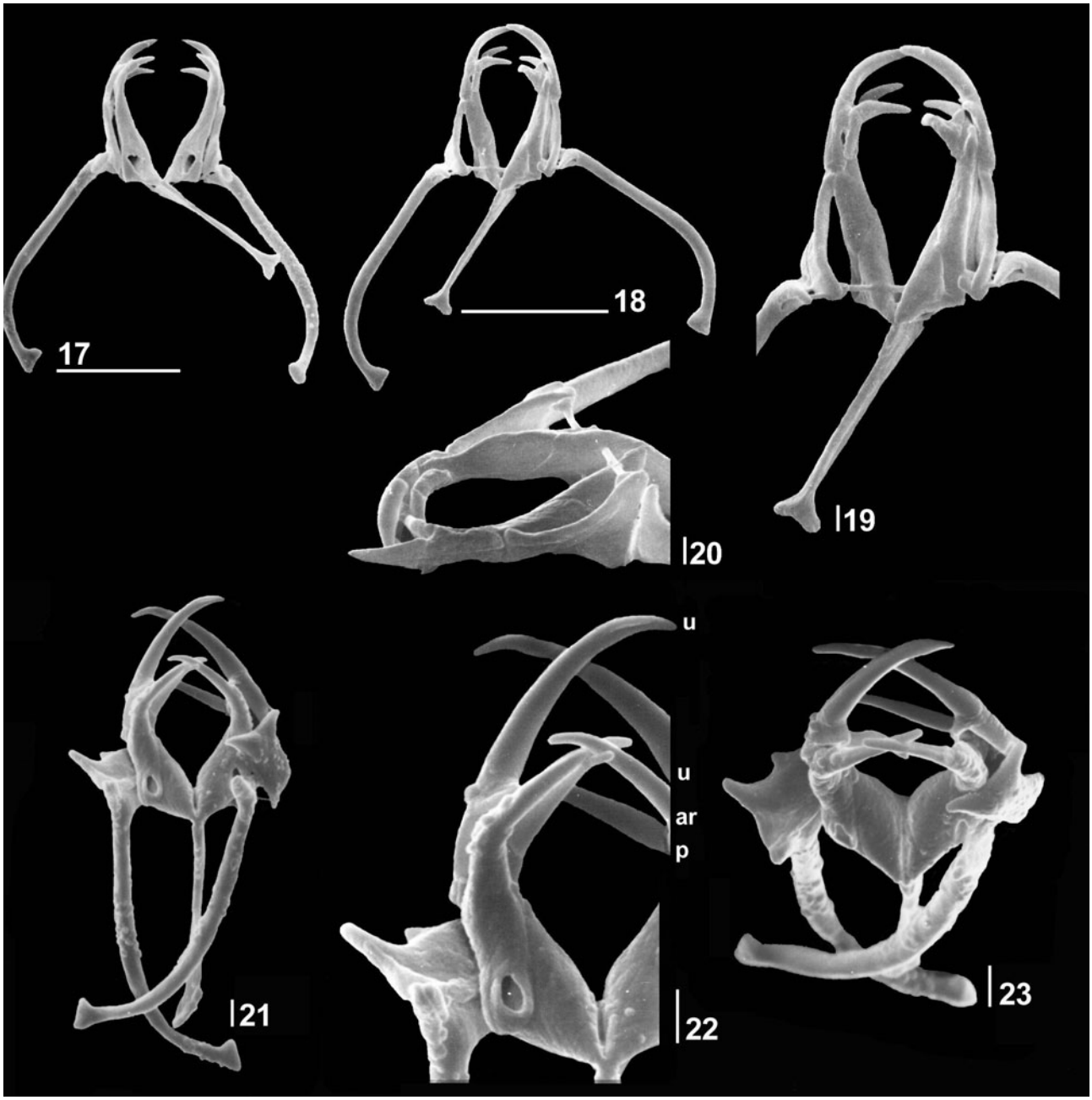
Measurements: Total length 137-160, toe 14-17, trophi 23-27. Ramus 9-10, fulcrum 10-11, uncus 4.4-5.9, intramalleus 5.2-5.5, manubrium 16.3-17.

Distribution and ecology

Encentrum pornsilpi n. sp. is so far known from its type locality only, where it occurred in abundance. It has not been found in the plankton.



Figs 3-5. – *Colurella psammophila* n. sp.. 3-4: lateral (different specimens), 5: ventral (ventral). – Fig. 6. – *Colurella colurus* f. *compressa*. – Figs 7-8. – *Encentrum longidens*. 7: lateral, 8: trophi, ventral. – Figs 9-10. – *Encentrum pornsilpi* n. sp.. 9: lateral, 10: ventral (corona contracted), 11: trophi, ventral. – Figs 12-16. – *Lepadella desmeti* n. sp.. 12: dorsal, 13: ventral, 14: lateral, 15: frontal, 16: caudal.



Figs 17-20. – *Encentrum pornsilpi* n. sp., SEM photographs of trophi. 17: dorsal, 18: ventral, 19: ventral, detail, 20: lateral.
Figs 21-23. – *Encentrum longidens*, SEM photographs of trophi. 21: dorsal, 22: dorsal, detail (p: preuncinal, ar: apical ramus, u: uncus tooth), 23: frontal.
Scale bars: 10µm (Figs 17, 18), 1µm (Figs 19-23).

Etymology: The species is named after Dr. Pornsilp Pholpunthin of PSU, Hat Yai, Thailand, in recognition of his contribution to the knowledge on the Thai rotifer fauna.

***Encentrum longidens* Donner, 1943**
(Figs 7, 8, 21-23)

Donner (1943) p. 70-71 figs 6a-e; De Smet & Pourriot (1997) p. 158 figs 423-427.

Comment

This small *Encentrum* is unmistakable by its general body shape and trophi with long, slender and weakly curved preunci, unci and apical rami teeth, and relatively long fulcrum. To date, the species is known from the type locality in Slovakia only (De Smet & Pourriot, 1997). As the report on the peculiar trophi of this small species is based on light-microscopic observation only and, consequently, lacks detail, we here present SEM photographs of this structure.

Lepadella desmeti new species

(Figs 12-16)

Material: Holotype in KBIN; one paratype in RUG, one in PSU. All collected in the hygropsammon of the type locality, Mai-Khao peat swamp, Phuket Island, Thailand, on 28 July 1999.

Differential diagnosis

The lorica shape of *Lepadella desmeti* n. sp. resembles a small *L. patella* (Müller, 1786), but these two species can be confused only if the ornamentation on the dorsal lorica of *Lepadella desmeti* n. sp. is overlooked. The new species is superficially similar to *L. rhodesiana* Wulfert, 1965. The latter has a relatively broader lorica, different head aperture (dorsal straight or only slightly concave, with punctated collar), and a foot in which the second foot pseudosegment is longest one (WULFERT, 1965).

Description

Parthenogenetic female (male unknown): Lorica stiff, relatively flat. Outline oval, with the greatest width in the posterior third, c. 1.5 times as long as wide. Dorsal plate convex, with two pairs of rounded longitudinal ridges, caudal end indented; a pair of openings to the lateral antenna present postero-laterally. Ventral plate weakly concave. Head aperture dorsally and ventrally concave, dorsally broadly U-shaped, ventrally deeper, V-shaped. No clear collar. Foot aperture squarish, longer than wide, lateral margins slightly diverging to posterior. Foot three pseudosegmented, two broad basal and one elongate and slender distal foot pseudosegment. Toes equal, straight (curved in the holotype, this probably an artefact), evenly narrowing to acutely pointed tips.

Measurements (of Bolivian specimen between brackets): Lorica length 72-78 (78), width 47-54 (48), head aperture width 21-25 (23), ventral sinus depth 15-18 (10), dorsal 6-10 (7), foot aperture width 14-17 (13), length 16-20 (22), toe length 21-25 (22), second foot pseudosegment length 5-6 (5), third 9-12 (9).

Distribution and ecology

Lepadella desmeti n. sp. occurred in low numbers in the interstitial of Mai-Khao peat swamp, but was not found during a previous study of the plankton of the swamp. However, a single specimen of what appears to be this species was recently reported from a plankton sample of a floodplain lake of the Ichilo River, Bolivia (SEGERS et al., 1998) . This indicates that, although the species is psammobiontic, it does occasionally leave the psammon for the plankton, like most other interstitial rotifers. Its present disjunct distribution may be an artefact of insufficient sampling of tropical interstitial habitats.

Etymology: The species is named after Prof. Dr. W.H. De Smet (RUCA, Antwerp), in recognition of his contributions to rotiferology.

ZOOGEOGRAPHY

Apart from the taxa treated above, the psammon rotifer community of Mai-Khao peat swamp contained three taxa of special zoogeographic interest. These are:

- *Colurella sanoamuangae* Chittapun et al., 1997 was only recently described from this peat-swamp, but is also known from a second locality on the Malay Peninsula, Lake Thale Noi (SEGERS & PHOLPUNTHIN, 1997). We found it in large numbers in the psammon, which indicates that it may be interstitial, intruding into the plankton only occasionally. Apparently, the species is endemic to Thailand.
- *Lecane acanthinula* (Hauer, 1938) is Oriental, although its range extends beyond the classical limits of this region (SEGERS, 1996).
- *Lecane segersi* Sanoamuang, 1996 was hitherto known from few specimens found at two localities in Northeast Thailand (SANOAMUANG & SEGERS, 1997), and occurred in high abundance in the psammon of Mai-Khoa peat swamp. Similarly to *C. sanoamuangae,* the species may be interstitial (psammophilous or psammobiontic), and is endemic to Thailand.

Of the 19 monogonont Rotifera found, four are regional endemics, one is an Oriental species (Table 1), and two are more widespread but very rare (*Encentrum longidens, Lepadella desmeti* n. sp.). Six taxa are new to Thailand. The interstitial rotifer fauna of the peat swamp has an endemicity rate of c. 20%. This seems high, notwithstanding that the total number of species on record is quite low (compare with DUMONT, 1983; SEGERS, 1996). However, the scarcity of information on interstitial rotifers, illustrated by the simultaneous description of one of the new species from Thailand and Bolivia, and the second record ever of *Encentrum longidens* after its description from Europe, illustrates that it is premature to generalise at this time.

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Electroreception of catfish *Ictalurus nebulosus* in uniform and non-uniform DC fields: detection threshold and body length

Lonneke B.M. Eeuwes, Robert C. Peters, Franklin Bretschneider and Wim J.G. Loos

Utrecht University, Comparative Physiology, Neuroethology
Padualaan 8, NL-3584 CH Utrecht, The Netherlands

ABSTRACT. Catfish are able to detect electric fields with their electroreceptor organs. It goes without saying that the electrodetection threshold depends on the sensitivity of the electroreceptor organs. The sensitivity in turn depends on a variety of extrinsic factors such as water temperature, conductivity, and electric field frequency. The aim of this study was to determine the effect of an intrinsic characteristic, namely body length, on the electrodetection threshold. In a two-alternatives forced-choice experiment, catfish of different sizes were tested in uniform or non-uniform direct-current fields. The results show no significant relation between body length and electrodetection threshold. The electrodetection thresholds are lower in uniform fields than in non-uniform fields. From this it is concluded that other factors than body size alone determine the electrodetection threshold.

KEY WORDS: electroreception, body length, catfish, behaviour, threshold.

INTRODUCTION

In the history of electrophysiology much research has been done to investigate if there exists a correlation between specimen size and the behavioural responses of fish to galvanic stimuli. In a variety of species, both electrosensitive and non-electrosensitive, the lowest current density that still elicited a response was found to increase with decreasing body length (ABE, 1935; SCHEMINZKY & SCHEMINZKY, 1931). Today, specimen size still plays a prominent part in research on electric fishing (STERNIN et al., 1972; ZALEVSKI, 1985). Surprisingly enough, in electroreception studies of the last five decades specimen size has not been taken into account, whereas a number of other factors on which the electrodetection threshold was thought to depend, such as ionic composition of the environment (PETERS et al., 1991; PETERS & WESTERINK, 1999; ROTH, 1971) electric field frequency, and temperature (PETERS et al., 1995a), were studied profoundly.

As catfish grow, the physiological properties of the electroreceptor organs change. Both the number of receptor cells and the number of ampullae per afferent nerve fibre increase, which causes not only an increasing signal-to-noise ratio and a sharpening of the bandpass filter but also an increase in the absolute sensitivity of the electroreceptor organ (PETERS et al., 1997; PETERS & IEPEREN, 1989, PETERS & MAST, 1983; TEUNIS et al., 1990; ZAKON, 1987). Moreover, a large specimen spans a larger area in the electric field and thus is able to perceive a higher potential difference than a small specimen (KALMIJN, 1974). On those grounds, a correlation between body length and the electrodetection threshold may be expected; a large specimen presumably has a lower electrodetection threshold than a smaller specimen.

Hence, behaviour that depends largely on the electric sense could differ between catfish of different sizes. Distances to prey at the initiation of an attack for instance, could, as in dogfish (KALMIJN, 1982), decrease with decreasing body length. The aim of the present study was to investigate in a psychophysical experiment if the sensitivity of catfish in direct current fields is size-dependent.

MATERIAL AND METHODS

Animals

Twenty-four specimens of freshwater catfish (*Ictalurus nebulosus*, LeSueur, 1819), eleven females and thirteen

Corresponding author: R. Peters, e-mail: R.C.Peters@bio.uu.nl

males, with weights ranging from 13 to 270 g and lengths of 95 to 280 mm, were subjects of this experiment. Juvenile catfish were obtained from Visplant (Numansdorp, The Netherlands). Several years prior to the experiments, adults were obtained from Van de Put (Zonhoven, Belgium). They were kept in glass tanks filled with tap water at Utrecht University until the experiments began. During the experiments the fish were kept in a glass aquarium, 91 x 30 cm, water height 10 cm. This aquarium was connected to a buffer tank, from which water was circulated and filtered. The total water volume was 180 litre. The tanks were placed in a climate-controlled room, and the temperature of the water was kept at 17 ± 2°C with a cooling device. Initially the tanks were filled with water, conductivity 0.31-0.38 mS/cm. The conductivity increased by approximately 0.01 mS/cm during the course of the experiments due to excretions of the fish and feeding. Once a week the water was partly refreshed. The fish were tested during the dark period of a 12h dark, 12h light regime. During the experiments the fish were fed minced beef with gelatine and agar-agar from peristaltic tubes on either side of the tank.

Working with small specimens (< 150 mm) required some adjustments: Water height was decreased by 2-4 cm to improve detection by the sensors used; the stimulus strength was adjusted. A small amount of Trouvit Elite response 1.6 mm (Paling, Putten, The Netherlands) was added to the food to make it more attractive for the juveniles, which were not used to eating beef. To avoid novelty stress, two juveniles were kept together in a test tank for a week before they were separated at the beginning of shaping.

Protocol

The electrodetection threshold was determined for each fish in a number of threshold sessions. The catfish were subjected to two sessions a night. A single session consisted of 100 or more trials. For the juveniles a single session consisted of 30 trials, because at that point they lost their appetite and motivation. At the beginning of each trial a light bulb above the test tank was switched on, which caused the fish to seek shelter underneath a PVC strip, approximately the same size of the fish, attached to the wall of the tank (Fig. 1A,B). If the fish stayed underneath its shelter for two seconds, the light was switched off and a weak uniform or non-uniform direct current field was presented. Each series of trials solely consisted of uniform field presentations or non-uniform field presentations. Each fish was subjected to only one form of field presentations.

In uniform fields, the side at which the anode was located was alternated pseudo-randomly. If the fish interrupted the infrared bundle nearest to the cathode, food was dispensed through the feeding tubes and 30 s of dark feeding time was offered. If the fish interrupted the infrared bundle nearest to the anode, the light bulb above the tank was switched on immediately and no food was offered.

In non-uniform fields the stimulus was presented either on the right or the left side. If the fish crossed the decision point at the side where the stimulus was presented, food was dispensed through the feeding tubes and 30 s of dark feeding time was offered. If the fish crossed the decision point at the opposite side, the light was switched on immediately and no food was offered.

At all times, the light above the test tank was operating as a negative reinforcer. After a correct choice, the strength of the following stimulus was decreased by 1 dB. After a false choice the following stimulus was increased by 3 dB (Fig.2). The steps up and down were not equal (respectively 3 dB and 1 dB) because if so, the stimulus would stay undetectable for a long period near the threshold value and the fish would become less motivated. This so-called staircase method eventually reveals the electrodetection threshold in orientation in catfish (Peters et al., 1995a).

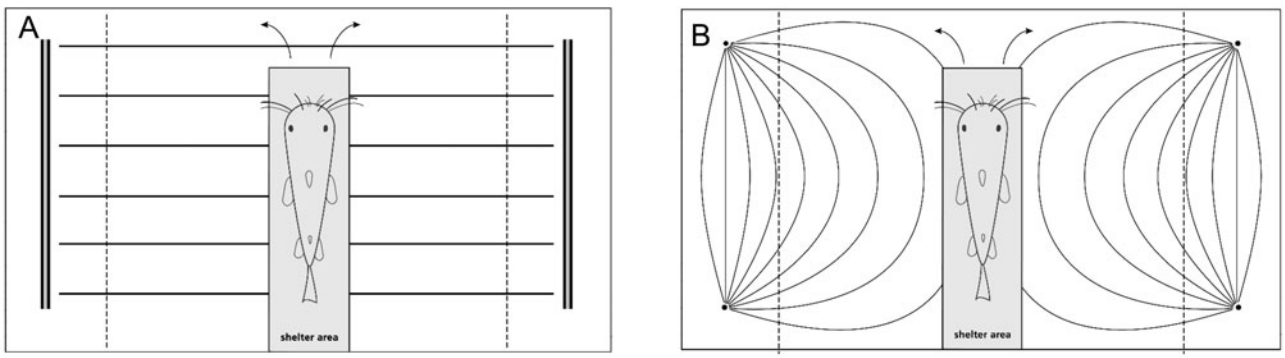

Fig. 1. – Schematic drawings of the experimental tanks used in uniform field presentation (A) or non-uniform field presentation (B), top view. The shelter area (grey) provides protection for top lights (not shown) and serves as a dwelling space between trials. Several centimetres from the electrodes infrared detectors are placed on the outside of the tank. At the same position a plastic bar (dotted line) is placed at the bottom of the tank to provide a tactile stimulus for the catfish. These bars are the decision lines. If the fish crosses a dotted line, it has made a choice. Food dispensers are placed between the dotted lines and electrodes. (A) Thick lines represent the strip electrodes. Parallel solid lines between electrodes represent the field lines during a trial. The polarity of the electrodes changes between trials. (B) Dots in the corners of the tank represent the steel bar electrodes. Solid lines between electrodes represent the field lines during a trial. The stimulus presentation is either on the left or the right side.

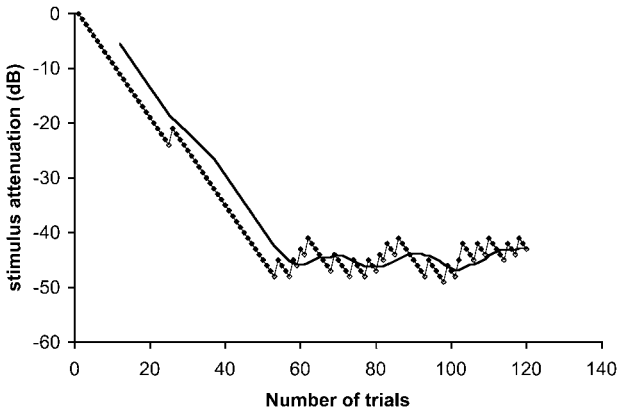

Fig. 2. – An original recording of the electrodetection threshold during a single session. At false choices the stimulus rises by +3 dB. The solid line without markers represents the running average over 12 successive trials. During analysis of data stimulus attenuation (dB) is converted into stimulus strength (μV/cm) and corrected for resistivity. In this example the electrodetection threshold is reached at -45 dB, which equals 0.72 μV/cm.

Stimulation

Since catfish can localise prey and orientate by means of their electroreceptor organs, dc dipole fields were used to mimic prey, and uniform direct-current (dc) fields to imitate environmental fields (PETERS & BRETSCHNEIDER, 1972). Stimuli were generated by a LAB-PC data acquisition card (National Instruments). The stimulus was fed into a home made voltage-to-current-converter (VCC), powered by floating power supplies. To generate uniform fields the VCC was connected to a pair of electrodes made of a strip of Perspex (15 x 30 cm) and silver wire. Creating a non-uniform field was achieved by means of two pairs of stainless steel bar electrodes with a diameter of 3 mm.

Shaping

Before the actual testing started, the fish was subjected to a period of shaping. In this period the stimulus protocols differed. In non-uniform fields the stimulus was presented right and left alternately, and in uniform fields the anode location was switched from one side to the other after each trial. The field strength (200-1000 mV/cm) was certainly within the perceptive range. As soon as the fish performed at a 90% level, the alternating left and right stimulus presentation in non-uniform fields and the anode location in uniform fields were randomised with a maximum of three in succession at the same side. When the level of correct choices was 90% or over, threshold determination was initiated. For the shaping period no particular skills are required, because the fish spontaneously orients to electric dipole sources, or to cathodes, and thereby autoshapes itself.

Statistics

Threshold has been defined as the minimum stimulus strength that could be detected by the fish for a certain period of time. As the steps up and down were unequal, every false choice had to be compensated for by three correct choices in order to maintain the same overall stimulus strength. To determine whether or not the catfish had reached its threshold or was still changing its performance, the running average over twelve successive trials was calculated. If the running average stayed the same for four successive calculations, this value was accepted as the threshold value. This means that the false-correct-correct-correct sequence had to be repeated at least three times, in which the order of the false and correct choices is irrelevant as long as the initiation point of the sequence is proceeded by more than three correct choices. If a single session yielded more than one threshold value, only the lowest was used in further analysis. Sessions that did not yield threshold values were left out. Kruskal-Wallis' non-parametric, distribution-free test for more than two independent samples was applied to detect differences in thresholds between catfish of varying size. A sample consisted of all threshold values found for a particular fish (with a certain body length). A regression analysis was also conducted.

RESULTS

Literature

Before the experiments were initiated a literature search was carried out to investigate what was already known about electrodetection thresholds. Over the years, all kinds of species have been subjected to research on detection of electric stimuli. To reduce the amount of information it yielded, the literature search was restricted to data of behavioural studies concerning fish. As shown in Fig. 3 and Table 1, a wide range of electrodetection

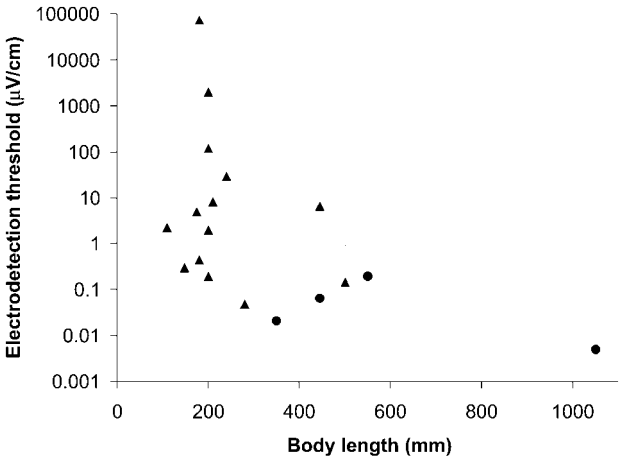

Fig. 3. – Relation between body length and electrodetection thresholds of 12 different electrosensitive species of fish. Freshwater species are represented by triangles, marine species by circles.
All values are taken or calculated from literature. Only data of behavioural studies is used. Although the experimental methods differ, almost all electrodetection thresholds are determined by a trained response. For three data points body length has been estimated by the author based on the average size of particular species and experiment devices used (open markers). For *Parasilurus asotus* the value given by ASANO & HANYU (1987) represents the real electrodetection threshold. See also Table 1.

thresholds ($5*10^{-3}$ to $7.5*10^{4}$ μV/cm) is covered by a relatively small range in body length (11-105 cm). In general, electrodetection thresholds are lower in marine species than in freshwater species. It should be noted, however, that most electrodetection thresholds published were the best ones found. Only rarely, body length of the particular specimen that produced this threshold was mentioned. If it was not mentioned, average body length of all specimens subjected to a certain study has been used.

TABLE 1

Relation between body length and electrodetection thresholds of 12 different electrosensitive species of fish. Freshwater species are represented by triangles, marine species by circles. See also fig. 3.

Body length (cm)	Species	Freshwater or Marine	Threshold (μV/cm)	Author
11	*Ictalurus nebuolosus*	F	2.25	Baranyuk (1981)
14.8	*Apteronotus albifrons*	F	0.3	Knudsen (1974)
17.5	*Ictalurus nebuolosus*	F	5	Peters et al. (1991)
18	*Parasilurus asotus*	F	75000	Abe (1935)
18.1	*Apteronotus albifrons*	F	0.45	Knudsen (1974)
20	*Ictalurus nebulosus*	F	2	Peters & Van Wijland (1974)
20	*Parasilurus asotus*	F	2000	Kokubo (1934)
20	*Potamotrygon*	F	120	Szabo et al. (1972)
20	*Sternarchus albifrons*	F	0.2	Granath et al. (1967)
21	*Ictalurus nebuolosus*	F	8.5	Peters et al. (1995b)
24	*Ictalurus nebuolosus*	F	30	Dijkgraaf (1968)
25	*Urolophus halleri*	M	0.005	Kalmijn (1982)
28	*Parasilurus asotus*	F	0.05	Asano & Hanyu (1987)
30	*Scyliorhinus canicula*	M	0.1	Dijkgraaf & Kalmijn (1962)
35	*Mustelus canis*	M	0.021	Kalmijn (1982)
44.5	*Anguilla rostrata*	F	6.7	Rommel & McCleave (1972)
44.5	*Anguilla rostrata*	M	0.067	Rommel & McCleave (1972)
50	*Clarias*	F	0.75	Lissmann & Machin (1963)
50	*Gymnarchus niloticus*	F	0.15	Machin & Lissmann (1960)
55	*Hydrogalus colliei*	M	0.2	Fields et al. (1993)
105	*Mustelus canis*	M	0.005	Kalmijn (1982)

General performance

Occasionally, a fish just did not respond during a trial or failed to make a decision in time. These so-called ”nogo” measurements are left out from the determination of the electrodetection threshold, because they rendered an artificial stabilisation of field strength. Reaction times, viz. time between onset of stimulus and choice, were measured during the entire experimental period. Both the number of “nogo” measurements and the reaction times were of use in the evaluation of the well-being of the fish.

Some of the catfish used in experiments with non-uniform field presentations were transferred to a second experimental tank, tested, transferred back to the first experimental tank and tested again. This was done to ensure that the differences between thresholds of the subjects were not due to the specific tank in which they were tested. No significant differences between tanks were detected, although in general, the catfish improved their performance over time.

Uniform fields

Electrodetection thresholds of six subjects are plotted against their body length in fig.4 (circles). Each data point is the average threshold calculated over at least seven and at most thirty-four threshold values found. Kruskal-Wallis’ test proved the smallest specimen used (110 mm) to have a significant higher threshold than only one of the larger catfish (240 mm). A second specimen of medium length (210 mm) was found to have a higher threshold than all specimens used except the smallest. No other significant differences were found. The correlation between electrodetection threshold and body length is not significant.

Non-uniform fields

Electrodetection thresholds of eighteen subjects are plotted against their body length in Fig.4 (open markers). Each data point represents the average threshold calculated over at least eleven and at most 180 threshold values

found. Statistical analysis confirms the observation that although electrodetection thresholds of subjects differ from each other, there is no significant correlation between electrodetection threshold and body length.

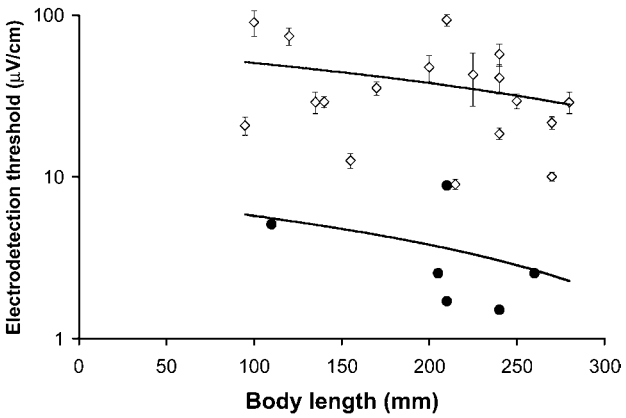

Fig. 4. – Electrodetection thresholds in relation with body length for catfish, *Ictalurus nebulosus*, in uniform fields (circles) and non-uniform fields (open markers). Each point corresponds with the mean threshold value plus S.E.M. of a specimen. For some specimens in uniform fields the S.E.M. falls within the marker. Thick solid lines are linear trendlines. The variation between the different individuals might mask a weak correlation between electrodetection threshold and body length. There is no significant correlation or regression.

DISCUSSION

In both uniform and non-uniform field presentations no significant correlation between body length and electrodetection threshold was found. It should be noted however, that threshold values in uniform fields were, on average, about ten times lower than threshold values in non-uniform fields. In part, this can be explained by the efficiency of the stimulus. In uniform fields the catfish moves parallel to the field lines, whereas in non-uniform fields the catfish moves in a certain angle with respect to the field lines. Therefore, in non-uniform fields, the potential cannot be measured across the entire body length. Thus, stimulation in uniform fields will be more effective than stimulation in non-uniform fields.

Although it does not show in the final data, occasionally it was observed that even the smallest specimen used in uniform fields could detect gradients as weak as 1µV/cm. As the convergence of the electroreceptor organs onto afferent nerve fibres increase during growth, small specimens have a lower signal-to-noise ratio. As a consequence, the electrodetection threshold could show more variability in small specimens. This could result in an overall higher threshold for small specimens, as changes in stimulus strength after each choice (steps up and down, Fig. 2) were biased.

In the experimental set-up, one major imperfection occurred. Although the side at which the presentation of stimuli was located alternated pseudo-randomly within a single session, every single session was exactly the same as the preceding. In theory, the fish could have learned the successive turns it had to make. This could explain the improvement of all catfish during the first days of the experimental period. On the other hand, when the session was initiated with a very low stimulus strength (well beyond the electrodetection threshold) the catfish made almost hundred percent false choices until the stimulus strength exceeded a certain level.

As the results indicate that total body length is not correlated with electrodetection thresholds, the expectations mentioned in the introduction have to be reconsidered. Information of the electroreceptors throughout the body might not be integrated in the higher order neurones as expected. Furthermore, the internal body of the catfish might not be as electrically uniform as assumed. In that case the internal reference potential cannot be considered as the mean potential between two equipotential lines perpendicular to the body axis of the fish. As a result, the actual internal reference potential(s) might be independent of the body length.

From this it is concluded that the relation between electrodetection threshold and body size is more complex than initially expected. Perhaps the temporal interactions between fish and electric field, as well as neural processing, are more important determinants for the electrodetection threshold than body length.

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Differences in geographic distribution and habitat of some cryptic species in the *Pardosa lugubris* group (Lycosidae, Araneae) in Belgium

Frederik Hendrickx [1], Katrien De Cock [1], Domir De Bakker [2] and Jean-Pierre Maelfait [1, 3]

[1] **Department Biology, Laboratory of Animal Ecology**
University of Ghent, K.L. Ledeganckstraat 35, B-9000 Gent
[2] **Department Entomology, Royal Belgian Institute of Natural Sciences**
Vautierstraat 29, B-1040 Brussel
[3] **Institute of Nature Conservation**
Kliniekstraat 25, B-1070 Brussel

ABSTRACT. The habitat and distribution of some closely related species of the *P. lugubris* s.l. group in Belgium are described to contribute to our understanding of the coexistence and speciation of these 'cryptic' species. With a few exceptions, *P. lugubris* has its main distribution in the lower part of Belgium where it occurs on sandy, nutrient poor soils. *P. saltans* occurs widely in Belgium except for in the Campine region where the species is totally absent. *P. alacris* was only found at three localities where limestone outcrops are present. The habitat of *P. lugubris* is pine and birch forests while in *Fagus* woodlands, only *P. saltans* was found. In *Quercus* forests, both species were found, often in mixed populations. A combination of micro- and macroclimatological features and habitat characteristics cause the differences in distribution of these species.

KEY WORDS: *Pardosa lugubris*, habitat, distribution, related species, coexistence.

INTRODUCTION

The lycosid spider formerly described as *Pardosa lugubris* s.l. (Walckenaer, 1802) comprises a complex of different species, of which the taxonomical status has been quite unclear for a long period (WÜNDERLICH, 1984; TÖPFER-HOFMANN & VON HELVERSEN, 1990; KRONESTEDT, 1992; KRONESTEDT, 1999; TÖPFER-HOFMANN et al., 2000). Their almost identical appearance and very similar genital organs are responsible for that unclear taxonomy. The shape and colour pattern of the male palpal cymbium are the most reliable traits to determine the species. Observational studies of the courtship display of the males showed that this behaviour contains the most distinct differences between the species. Such species are often called "cryptic" species. The courtship display is interpreted as the most important species-barrier, as cross species mating tests in the laboratory revealed that females never accepted a heterospecific male (TÖPFER-HOFMANN & VON HELVERSEN, 1990). At present, six different species of the *P. lugubris* group are formally described: *Pardosa lugubris* s.s. (Walckenaer, 1802), *P. saltans* Töpfer-Hofmann, 2000, *P. alacris* (C.L. Koch, 1833), *P. baerhorum* Kronestedt, 1999, *P. pertinax* von Helversen, 2000 and *P. caucasica* Ovtsharenko, 1979.

Data about the differences in habitat preference and the distribution patterns of these species are still very rare as they were not treated as distinct species in the past. Former records of the habitat preference of *P. lugubris* s.l. in Belgium are light forests and forest edges (ALDERWEIRELDT & MAELFAIT, 1990), and no bimodality or gradient in habitat preference was observed, nor was any heterogeneity found in its distribution. Furthermore, studies that distinguish the different species (DE BAKKER, 1998; TÖPFER-HOFMANN et al., 2000) note that many species live in the same habitat, occupy the same activity pattern and phenology and that combinations of two or even three different species can be found syntopically in mixed populations. This poses the problem of the coexis-

Corresponding author: F. Hendrickx,
e-mail: frederick.hendrickx @rug.ac.be

tence and the speciation of these extremely similar species.

Therefore, detailed information about co-occurrence, habitat preference and distribution patterns of the different members of the *P. lugubris* group may reveal if they are "ecologically relevant" species and provide an acceptable explanation for the speciation in the past.

Secondly, we want to investigate which of the different species occur in Belgium. Of the four species in the *Pardosa lugubris* group, only the occurrence of *P. alacris* and *P. lugubris* s.l. has been confirmed until now (ALDERWEIRELDT & MAELFAIT, 1990).

MATERIAL AND METHODS

Geographic distribution patterns

Information about the distribution patterns originated from hand sampling and the results of a research project investigating forest soil arthropod fauna in which 56 different forest plots, evenly distributed over Flanders, were sampled with three pitfalls each during a complete year cycle (see DE BAKKER et al., 1998 for more details), museum collections of the Royal Belgian Institute of Natural Sciences and private collections of M. Janssen, D. Bonte and the first author.

Hand sampling was conducted in the period when adult males are active, from the second half of April till the first half of May in 1999. Because of the unreliability of the determination of the females, males were captured at 20 randomly selected locations (at least 20 males per location). They were kept in alcohol afterwards and determined following Topfer-Hoffman et al, 2000.

The second set of data was obtained from the research project in which 56 different forest plots were sampled. Data from the 25 plots where a species of the *P. lugubris* group was captured were used to analyse the distribution pattern.

Additional data were obtained from re-examination of the material determined as *P. lugubris* s.l. in collections of the Royal Belgian Institute of Natural Sciences and some private collections.

The distribution of each species is presented on a map that shows UTM squares (10 km x 10 km). Maps were created with ArcView version 3.1. Association with soil type was determined by analysing an overlay with a soil map of Flanders.

Habitat characteristics

A description of the habitat was made by estimating the cover (%) of tree, scrub and herb layer. For each layer, the percentage cover of each plant species was estimated in a 10 m x 10 m area encompassing the site where the species were caught. Characterisation of the habitat is based on the composition of the vegetation, the depth of the soil litter layer and the percentage of ground covered by death wood. Data about the habitat characteristics were only available from the above mentioned hand sampling campaign and the research project investigating forest soil arthropod fauna (DE BAKKER, 1998).

A Detrended Correspondence Analysis (DCA) (HILL & GAUGH, 1980) of the vegetation samples was conducted to investigate whether the main factors influencing the composition of the vegetation are also reflected in the occurrence of the different members of the *P. lugubris* group.

To test whether particular plant species are significantly more associated with the occurrence of a certain species, indicator values were calculated for each plant species. Therefore, all vegetation samples were divided into two groups (one for *P. lugubris* and one for *P. saltans*) and the indicator value of each plant species was calculated. Mixed populations were not included in the analyses as we only wanted to specify which plant species are indicative for the habitat of one of the two spider species. As indicator value, we used the IndVal-value (DUFRÊNE & LEGENDRE, 1997), which combines the relative abundance of a species with its relative occurrence in the two groups. To test if a plant species had a significantly larger IndVal for one of the two groups, sites were randomly allocated (1000 times) between the two groups. Significance was evaluated as the rank of the observed value in the randomly generated distribution ordered in decreasing order.

RESULTS

Geographic distribution

In total 100 records about the distribution of the species in Belgium could be obtained. These were divided over 76 UTM squares (20% of all UTM squares of Belgium). Of the six species distinguished in the *P. lugubris* group, only three were found, namely *P. lugubris* s.s. (41 UTM squares), *P. saltans* (44 UTM squares) and *P. alacris* (3 UTM squares). No specimen showed morphological characters that are typical for *P. baerhorum, P. pertinax* or *P. caucasica*. Fig. 1 gives the distributions of the three species over UTM squares. Because the results of the distribution pattern were not related to the method of collection, all data are presented on the same map.

Although there is an overlap in the distribution of the species, some clear patterns can be distinguished. *P. lugubris* has its main distribution in the northern part of Belgium. With a few exceptions, it is almost completely absent south of the rivers Samber and Meuse. The two exceptional records where *P. lugubris* was found south of the rivers Samber and Meuse are both from very warm limestone habitats. The line formed by these two rivers separates the higher parts (200-600 m above sea level) in the south and the lower part (0-200 m above sea level) in the north. In the lower part, it occurs abundantly in the east, called the Campine region. This region is charac-

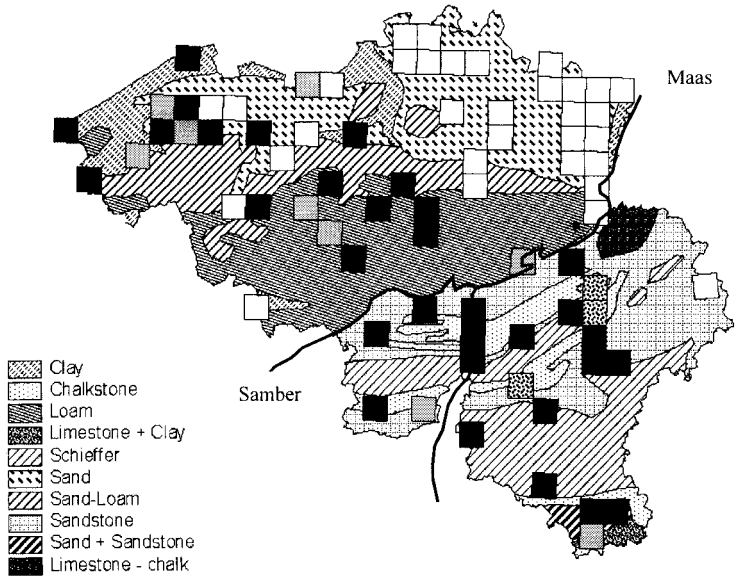

Fig. 1. – Overlay of the soil map of Belgium and the map showing 10 km x 10 km UTM-squares where the different members of *Pardosa lugubris* s.l. were found (open UTM squares = *P. lugubris* s.s.; black UTM squares = *P. saltans*; grey UTM squares = *P. lugubris* s.s. + *P. saltans*; dotted UTM squares = *P. saltans* + *P. alacris*)

terised by a relatively continental climate with stronger winters and hotter summers, compared with the western part of Belgium (ALEXANDRE et al., 1992). The soil consists in origin of very nutrient poor and acid sandy soils. An overlay with the main soil types of the northern part of Belgium is plotted on the distribution map in Fig. 1.

In contrast with *P. lugubris*, *P. saltans* appears to be totally absent on the sandy soils of the Campine region in the north-eastern part of Belgium. Its main distribution is located south of the rivers Samber and Maas. North of this line, it can be found on the loamy and sandy loam soils, along the coast, and on the sandy soils in the western part of Flanders.

The third species, *P. alacris* was found to be very rare in Belgium. There were only three locations, all with limestone outcrops originating from the central Devon (south) and the early Carbon (north).

Habitat

No detailed habitat descriptions are available for *P. alacris.* The three known populations occurring in southern Belgium were all found along forest edges on rocky limestone slopes exposed to the south. One of those three populations was mixed with *P. saltans*.

In total 45 plant species lists were available, 24 of *P. lugubris* populations, 16 for *P. saltans* and five for sites in which the two species co-occurred. There were no significant differences (*t*-test; $p > 0.05$) between the litter depths and the estimated quantities of dead wood for the two species. Localities where *P. lugubris* was captured showed a much higher cover of mosses, mainly species of the genus *Polytrichium*.

An indirect gradient analysis (DCA) on the basis of the estimated percentage ground cover of the plant species making up the herb (h), scrub (s) and tree (t) layer results in the ordination shown in Fig. 2.

Along the first axis (Eigenvalue: 0.822), *P. lugubris* is present along the whole gradient while *P. saltans* on the other hand is restricted mainly to the left side of the axis. The dominant plant species reveal that this gradient goes (from left to right) from *Fagus*-woodlands and *Quercus* (mainly *Quercus robur*) forests over *Betula pendula* forests to *Pinus sylvestris* woodlands. These forest types indicate that there is a gradient of less nutrient poor, lightly acidic soils (*Quercus robur* and to a lesser extent *Fagus sylvaticus*) towards very acid, nutrient poor sandy soils (*Pinus sylvestris*) (ELLENBERG, 1979; VANDENKERKHOVE, 1998; STORTELDER et al., 1999). There are two exceptional populations, indicated in Fig. 2 as "Meer3" and "Gulke" where *P. saltans* was captured in a birch forest. Location "Meer3" was, however, a very small birch forest of only one hectare that is totally enclosed in a large beech and oak forest. Other samples originating from the same forest, indicated as "Meer1" and "Meer2" in Fig. 2, show that they have a vegetation composition which fits well into the *P. saltans* habitat. In the population "Gulke", situated in the western part of Flanders, only a few individuals of *P. saltans* were found amongst individuals of *P. lugubris*. The individuals were captured in a clear-cut area with young shoots of birch trees of only one meter in height.

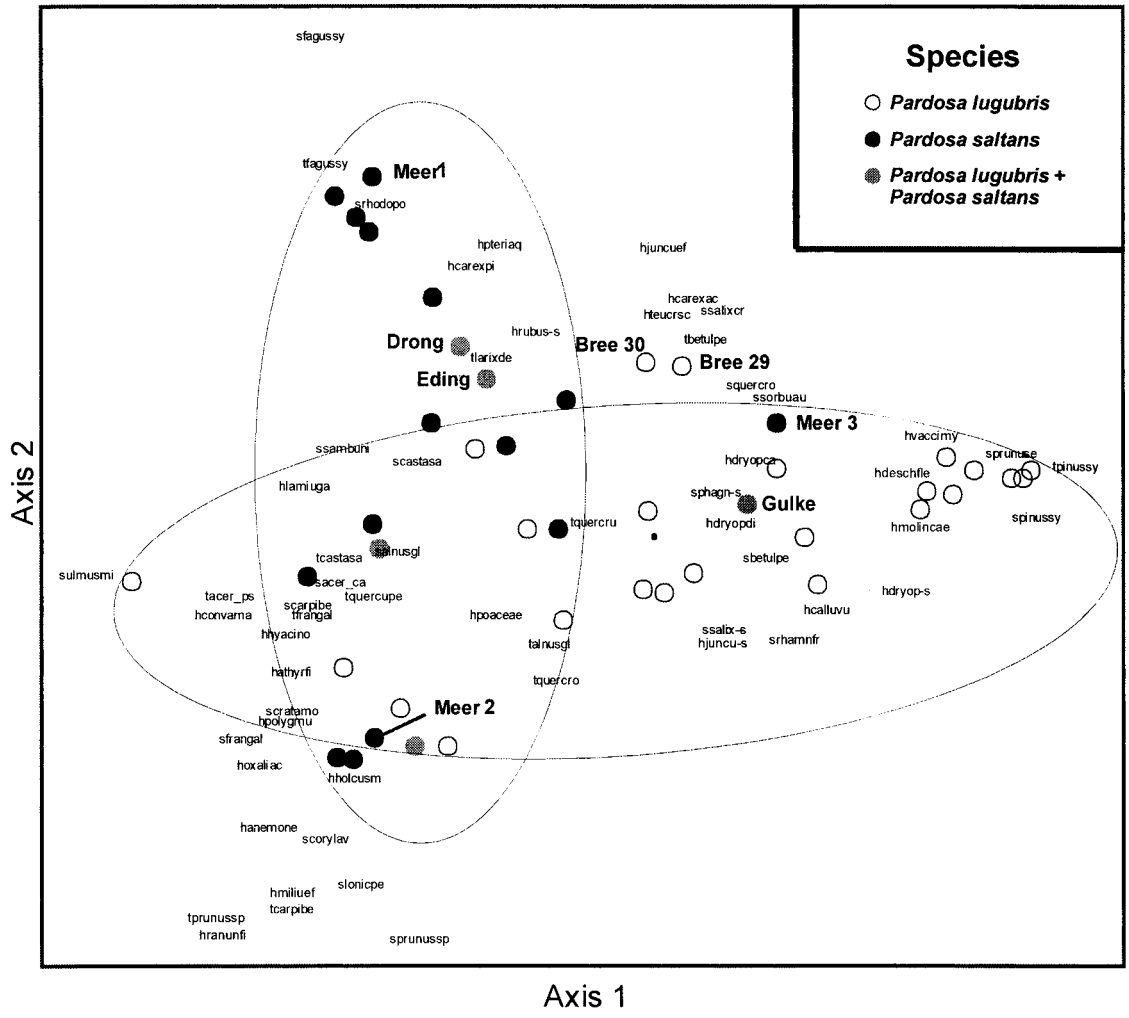

Fig. 2. – DCA-analysis of the 45 sites based on the vegetation composition. Points represent the presence of *P. lugubris* and/or *P. saltans.* Plant species are abbreviated according to the layer where they were present (h= herb; s = scrub, t = tree) followed by the first five characters of the genus and the first two characters of the species name.

Along the second axis (Eigenvalue: 0.611), *P. saltans* is present along the whole gradient, while *P. lugubris* is restricted to the lower part. Populations on the upper part of the graph were captured in forests where *Fagus sylvaticus* was the dominant tree species, while *Quercus robur* forests are located on the lower part of the axis. Also for *P. lugubris*, there are a few exceptional populations, indicated in Fig. 2. "Bree29" and "Bree30" are from the same forest with a vegetation which was a bit more *P.saltans*-like. This forest is situated in the Campine region in the north-east of Flanders. In "Drong" and "Eding", respectively situated in the loamy sand and the loamy region.

The remaining populations, where both species occur syntopically, are located towards the centre of the ordination of the vegetation samples, where *P. lugubris* as well as *P. saltans* occurs.

Table 1 lists the names of the most encountered plant species (encountered in more than 8% of the investigated sites). They are ordered from highest IndVal for *P. lugubris* towards highest IndVal for *P. saltans*. *Pinus sylvestris* (tree-layer), *Prunus serotina* and *Betula pendula* (scrub-layer) and *Molinia caerulea* (herb-layer) have a significantly larger indicator value for *P. lugubris*. All are indicative for nutrient poor, acid and sandy soils. For *P. saltans*, *Fagus sylvatica* (tree-layer), *Carpinus betulus*, *Cratageus monogyna* and *Fagus sylvatica* (scrub-layer) and *Anemone nemorosa* (herb-layer) have a significantly larger indicator value. Tree species such as *Quercus robur*, *Rubus*-sp. and *Sorbus aucuparia* were encountered at more than 25% of the investigated sites, and showed no higher correlation with one or the other of the two spider habitats.

TABLE 1

List of most abundant plant species ordered from highest IndVal for *P. lugubris* (top) to highest IndVal for *P. saltans* (bottom) (IndVal= Indicator Value following Dufrêne & Legendre, 1997; p-value = level of significance that plant species is indicative for *P. lugubris* or *P. saltans* habitat; total # sites = number of sites where plant species was observed; % of total # sites = total number of sites where the plant species was observed).

***Plant species associated with* P. lugubris**				
Species	*IndVal*	*p-value*	*total # sites*	*% of total # sites*
Betula pendula (scrub)	57,35	0,002	13	29
Pinus sylvestris (tree)	45,83	0,001	6	13
Prunus serotina (scrub)	40,5	0,047	11	24
Molinia caerulea (herb)	35,25	0,048	7	16
Sorbus aucuparia (scrub)	30,92	0,326	14	31
Deschampsia flexuosa (herb)	26,99	0,205	8	18
Quercus robur (tree)	26,3	0,635	13	29
Vaccinium myrtilum (herb)	25	0,055	5	11
***Plant species associated with* P. saltans**				
Species	*IndVal*	*p-value*	*total # sites*	*% of total # sites*
Fagus sylvatica (tree)	53,22	0,001	8	18
Rubus-sp. (herb)	46,21	0,153	17	38
Fagus sylvatica (scrub)	30,67	0,009	4	9
Anemone nemorosa (herb)	27,37	0,023	5	11
Acer pseudoplatanus (scrub)	26,25	0,137	9	20
Cratageus monogyna (scrub)	25	0,014	4	9
Carpinus betulus (scrub)	25	0,021	4	8
Lonicera periclimenum (srub)	21,67	0,148	4	9
Quercus rubra (tree)	20,57	0,344	6	13
Corylus avellana (scrub)	19,83	0,244	9	20
Pteridium aquilinum (herb)	19,62	0,244	6	13
Hyacinthoides non-scripta (herb)	16,42	0,162	6	13
Betula pendula (tree)	14,62	0,895	8	18

DISCUSSION

Although *P. saltans*, *P. lugubris* and *P. alacris* were sometimes found together in mixed populations, each species, however, seems to have a different optimal habitat, which may explain the difference found in their geographic distribution

The distribution map of *P. lugubris* shows that this species is almost entirely restricted to the sandy soils in the lower part of Belgium. Besides the lower nutrient content of the soil and the vegetation composition, this region is also characterised by a warmer climate during the summer months. This is in part due to the faster warming up of the upper soil layer compared with clay and loamy soils. In the Campine region, located in the eastern part of Flanders, this is even more pronounced because of the lesser cooling influence of the sea (ALEXANDRE et al., 1992). The average temperature south of the rivers Samber and Maas is lower compared to the lower part of Belgium, which could explain the absence of *P. lugubris* in this region. The two exceptional locations where *P. lugubris* was found south of this line, are characterised by a warm microclimate (ALEXANDRE et al., 1992). Therefore *P. lugubris* seems to be a more thermophilous species than *P. saltans*.

The habitats of *P. lugubris* and *P. saltans* can be divided into three main groups. Firstly, a group where *P. lugubris* occurs alone where typical plant species are *Pinus sylvestris*, *Molinia caerulea*, *Prunus serotina* and *Betula pendula*. Fytosociologically, forests with such a plant composition are assigned to *Vaccinio-Piceetea* (STORTELDER et al., 1999). It is a forest type that is typical for acid and sandy, nutrient poor environments. Most of them are not the original forest type but were planted at the beginning of the 20th century on former heathlands. Forests where *P. lugubris* as *P.saltans* can also be found are mostly dominated by *Quercus robur*, *Quercus petrea*, *Rubus* sp., *Castanea sativa* and *Sorbus aucuparia*. This forest type, called *Quercetea robori-petraeae* (STORTELDER et al., 1999) develops on sandy to sandy loam soils. They are more nutrient rich and less acidic than *Vaccinio-Piceetea* soils (VANDEKERKHOVE, 1998). Sites where *P.*

saltans occurs alone are mainly forests dominated by *Fagus sylvatica*, *Quercus robur*, *Carpinus betulus* and have *Anemone nemorosa* in the undergrowth (*Querco-Fagetea*). This is a quite old forest type, which needs about 100-300 years to develop and has its main distribution on the loamy soils of Belgium. The three records of *P. alacris* seem to indicate that the species is possibly restricted in Belgium to forest edges on limestone.

The results presented here indicate that each of the three represented species of the *P. lugubris* group has its own distinct habitat optimum. A combination of several factors such a micro- and macroclimatological features and habitat characteristics causes the differences in distribution between the different members of the *P. lugubris* group.

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Localization of pyrokinin-like immunoreactivity in the brain of the crayfish *Astacus leptodactylus* (Crustacea)

Pieter Torfs, Inge Mertens, Joris Vriens, Arnold De Loof and Liliane Schoofs

Zoological Institute, Katholieke Universiteit Leuven, Naamsestraat 59, B-3000 Leuven (Belgium)

ABSTRACT. Pyrokinins are members of the pyrokinin/PBAN family of neuropeptides, which have been found in many insect species, and recently also in the crustacean *Litopenaeus vannamei*. Members of this family regulate reproductive processes in insects, including pheromone biosynthesis. Pyrokinin-like immunoreactivity has been studied in insects, but not in crustaceans. In this study, a polyclonal antibody against pyrokinin 1 of *Litopenaeus vannamei* was used to demonstrate the presence of pyrokinin-like material in the brain of the crayfish *Astacus leptodactylus*. Immunoreactivity was found in cells of the tritocerebrum, indicating that pyrokinin-like peptides are present in crayfish.

KEY WORDS: Pyrokinin, immunocytochemistry, neuropeptide, Crustacea.

INTRODUCTION

Pyrokinins are members of the pyrokinin/PBAN pheromone biosynthesis activating neuropeptide family of neuropeptides, which have been found to be responsible for a variety of functions in invertebrates such as myotropic activity of the hindgut in cockroach (NACHMAN et al., 1986), locust (SCHOOFS et al., 1991) and crayfish (TORFS et al., 2001), and stimulation of sex pheromone biosynthesis (GAZIT et al., 1990). Members of this family are characterized by the C-terminal amino acid sequence FXPRLamide. Pyrokinins (PK) are characterized by this typical C-terminus and by the fact that they were isolated through their myotropic activity. To date, 17 members of the pyrokinin subfamily have been characterized (Table 1). They were first isolated from the cockroach *Leucophaea maderae* (HOLMAN et al., 1986). Subsequently, pyrokinins were identified in the locusts *Locusta migratoria* (SCHOOFS et al., 1990a; 1990b; 1991, 1992b; 1993) and *Schistocerca gregaria* (VEELAERT et al., 1997), and the American cockroach *Periplaneta americana* (PREDEL et al., 1997, PREDEL et al., 1999; PREDEL & ECKERT, 2000). Most recently, our group has isolated the first members of this family from non-insects. Pev-PK 1 and Pev-PK 2 were isolated from the crustacean *Litopenaeus vannamei* through their ability to induce *L. maderae* hindgut contraction (TORFS et al., 2001). Both pyrokinins appear to be active at physiological concentrations on both insect (*L. maderae*) and crustacean (*Astacus leptodactylus* (Eschscholz, 1823)) hindgut.

The distribution of FXPRLamide-like immunoreactivity in the central nervous system of insect species has been investigated thoroughly (SCHOOFS et al., 1992a; TIPS et al., 1993; BRÄUNIG et al., 1996; PREDEL & ECKERT, 2000). Immunostaining in the brain was revealed in all investigated insect species. In crayfish, the presence of pyrokinin-like factors has not yet been reported. This paper reports the study of the distribution of pyrokinin-like immunoreactivity in the brain of the crayfish *Astacus leptodactylus*.

Corresponding author : P. Torfs, e-mail : pieter.torfs@bio.kuleuven.ac.be

TABLE 1

Sequence comparison of pyrokinins. Amino acids that are conserved throughout the pyrokinin subfamily are in boldface. pQ indicates a pyroglutamic acid residue.

Classis	Species	Peptide name	Sequence	Reference
Insecta	*L. maderae*	Lem-PK	pQTS**FT**P**RL**-**NH$_2$**	HOLMAN et al., 1986
	L. migratoria	Lom-PK I	pQDSGDEWPQQP**F**V**PRL**-**NH$_2$**	SCHOOFS et al., 1991
		Lom-PK II	pQSVPT**FT**P**RL**-**NH$_2$**	SCHOOFS et al., 1993
		Lom-MT I	GAVPAAQW**FS**P**RL**-**NH$_2$**	SCHOOFS et al., 1990a
		Lom-MT II	EGD**FT**P**RL**-**NH$_2$**	SCHOOFS et al., 1990b
		Lom-MT III	RQQP**F**V**PRL**-**NH$_2$**	SCHOOFS et al., 1992b
		Lom-MT IV	RLHQNGMP**FS**P**RL**-**NH$_2$**	SCHOOFS et al., 1992b
	S. gregaria	Scg-MT I	GAAPAAQ**FS**P**RL**-**NH$_2$**	VEELAERT et al., 1997
		Scg-MT II	TSSLFPH**PRL**-**NH$_2$**	VEELAERT et al., 1997
	P. americana	Pea-PK-1	HTAG**FI**P**RL**-**NH$_2$**	PREDEL et al., 1997
		Pea-PK-2	SPP**FA**P**RL**-**NH$_2$**	PREDEL et al., 1997
		Pea-PK-3	LVP**FR**P**RL**-**NH$_2$**	PREDEL et al., 1999
		Pea-PK-4	DHLPHVYS**PRL**-**NH$_2$**	PREDEL et al., 1999
		Pea-PK-5	GGGGSGETSGMW**FG**P**RL**-**NH$_2$**	PREDEL et al., 1999
		Pea-PK-6	SESEVPGMW**FG**P**RL**-**NH$_2$**	PREDEL & ECKERT, 2000
Crustacea	*L. vannamei*	Pev-PK 1	DFA**FS**P**RL**-**NH$_2$**	TORFS et al., 2001
		Pev-PK 2	ADFA**FN**P**RL**-**NH$_2$**	TORFS et al., 2001

MATERIAL AND METHODS

Animals

Mature specimens of the crayfish *Astacus leptodactylus* were purchased from a local seafood dealer (Colette, Belgium). They were maintained in freshwater tanks where the water was recirculating constantly through sand filtration units. The crayfish were kept at room temperature. Animals were anaesthetized by packing in ice for 30 min.

Production of antiserum

Synthetic Pev-PK 1 was coupled through the free carboxyl group of the N-terminal aspartate residue to bovine thyroglobulin using EDC (1-ethyl-3-(dimethylaminopropyl)carbodiimide hydrochloride). In this way, antisera against thyroglobulin bound DFAFSPRL-NH_2 would be mainly directed against the C-terminal portion of the peptide molecule. After overnight incubation, the water-soluble isourea, which is released as a by-product of the conjugation reaction, and the excess reagent were separated from the hapten-carrier complex by dialysis. The complex was dissolved in distilled water and emulsified with an equal amount of Freund's complete adjuvant and injected subcutaneously into New Zealand white rabbits. Second, third and fourth boosts were given, using Freund's incomplete adjuvant, respectively two, four and six weeks after initial immunisation. The antiserum was characterized using immuno-dot-blot according to SALZET et al., 1997.

Immunocytochemistry

The brain was dissected in Bouin-Hollande's (10%) sublimate fixative. After 18 to 24 hr of fixation, the brains were rinsed in distilled water (12hr), dehydrated in an ethanol series (70, 95 and 100% for two times two hrs), cleared in Histosol plus and embedded in Paraplast. Alternating sections of 4 µm were made with an LKB Historange microtome using glass knives. The sections were processed using the peroxidase-antiperoxidase method (VANDESANDE & DIERICKX, 1976) with 3,3' diamino-benzidine as the peroxidase substrate. Method specificity was controlled by application of the preimmune rabbit antiserum taken from the same animal that produced the primary antiserum. Serum specificity was determined by absorption of the antiserum with the antigenic determinant it was directed against, i.e. Pev-PK 1.

RESULTS AND DISCUSSION

Characterization by immuno-dot-blot revealed that the produced antiserum, used in a dilution of 1/1000, recognized synthetic Pev-PK 1 (to which it was raised) and synthetic Pev-PK 2. Seven to eight tritocerebral cell bodies were labelled with anti-Pev-PK 1 (Fig. 1, top). No staining was observed when the anti-Pev-PK 1 serum previously inactivated with Pev-PK 1 was used, nor with control serum of the preimmunized rabbit (Fig. 1, bottom). Comparable results were obtained in the brain of several insect species. In *L. migratoria*, *P. americana* and *L. maderae* cell bodies were visualized in the tritocerebrum (TIPS et al., 1993). Our results confirm that

pyrokinin-related peptides have a wider distribution in Arthropoda than has been thought.

Of the various known functions of FXPRLamide-containing peptides, only the myotropic one has been studied in crustaceans up to now (Torfs et al., 2001). Further research has to be undertaken to assess the physiological significance of these crustacean pyrokinins. In crustaceans, urine-born pheromones play an important role in sexual behaviour. Although previous studies suggest that sex pheromones produced by females are important in courtship (Atema & Engstrom, 1971), no female sex pheromones have been chemically identified to date. In contrast with insects, little is known about the endocrinological processes underlying this phenomenon in crustaceans. The fact that pyrokinin-like immunoreactivity is found in the brain of *A. leptodactylus* could give great impetus towards sex pheromone research in crayfish and even astaciculture. The latter is of particular interest lately in many European countries, being promoted for its promising commercial prospects (Pérez et al., 1997).

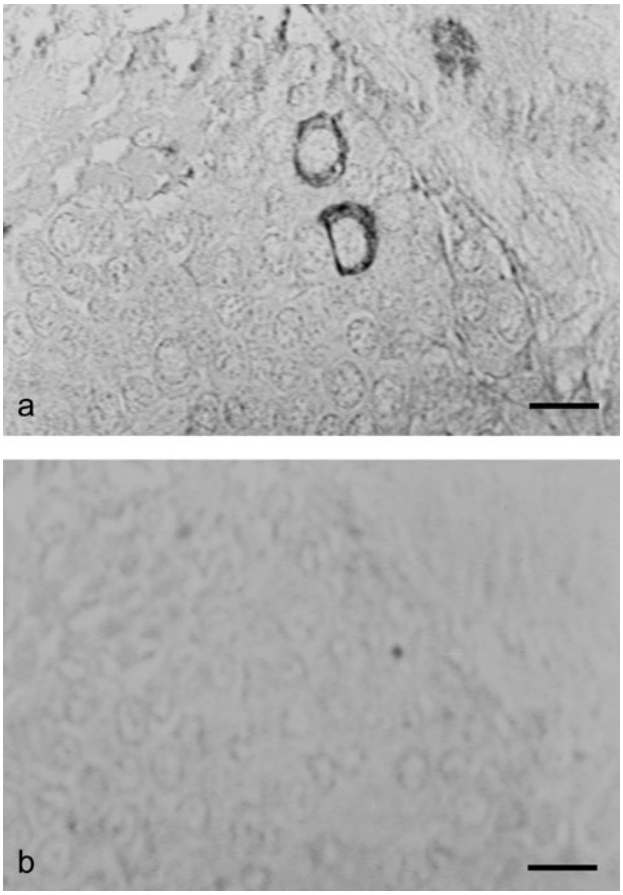

Fig. 1. – Pyrokinin-like immunoreactivity in the brain of *Astacus leptodactylus*. Top: Two immunostained cells in the tritocerebrum. Bottom: Control, alternate section showing no immunoreactivity with the serum of the preimmunized rabbit. Scale bar is 30 μm.

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Natural bioluminescence as a genetic marker for ophiuroid species

Samuel Dupont[1,2], Jérôme Mallefet[1] and Yannick Dewael[1]

[1]**Laboratoire de Physiologie Animale, Université catholique de Louvain, Bâtiment Carnoy, 5 Place Croix du Sud, B-1348 Louvain-la-Neuve, Belgium**
[2]**Centre de Recherche sur la Biodiversité, Université catholique de Louvain, Bâtiment Carnoy, 5 Place Croix du Sud, B-1348 Louvain-la-Neuve, Belgium**

ABSTRACT. Bioluminescence is the emission of visible light by living organisms. This amazing property is used in various research fields such as genetics, molecular biology, chemistry, etc. The aim of this work was to gather evidence that bioluminescence could also be used as a genetic marker in various luminescent species. Previous studies with the brittlestar *Amphipholis squamata* have shown that bioluminescence is an excellent marker for intraspecific genetic variability. The method, using maximum luminous capabilities induced by KCl 200mM depolarization, presents numerous advantages over other genetic markers (RAPDs, microsatellites, RFLP, etc.): it is cheaper, faster and easier to use. Since bioluminescence is a frequent phenomenon in brittlestars, the same method was used to compare variability between six different species: *Amphipholis squamata, Amphiura filiformis, A. arcystata, Ophiopsila aranea, O. californica* and a new species of *Amphiodia*. Our results show that *Amphiodia* and both *Ophiopsila* species could be clearly isolated, each in a separated cluster, according to their luminous capabilities. These differences could be explained by physiological properties. On the other hand, an important intraspecific variability was observed for two species (*A. squamata* and *A. filiformis*). We propose that natural bioluminescence is a good marker to study inter- and intraspecific variability, providing useful functional information for physiological and population studies.

KEYWORDS: Ophiuroidea, echinodermata, bioluminescence, phylogeny, biodiversity, population genetics, markers.

INTRODUCTION

Since the 19th century and the theory of evolution, natural scientists have been looking for individual variations within and between populations (RIDLEY, 1997). In order to understand the evolution of these variations, population biologists use different categories of genetic markers: morphological, biochemical or more recently molecular markers (SUNNUCKS, 2000). In the past decade, the contribution of molecular genetics to population biology has been huge. Many new genetic markers appeared with the development of the polymerase chain reaction (PCR) and the advent of routine DNA sequencing. Examination of these markers at the right scales of time, space and change can give information on the distribution and evolution of genetic variants. For example, microevolutionary processes such as migration, natural selection or reproductive success could be investigated using this method. Therefore, measuring genetic variations and the influence of the environment, we can make inferences on the biology of the species (SUNNUCKS, 2000; FÉRAL, 2000).

Bioluminescence is the capability of living organism to produce visible light (HASTINGS & MORIN, 1991). This amazing phenomenon is commonly observed in echinoderms where more than 40% of the luminous species are ophiuroids (HERRING, 1995; MALLEFET, 1999). Few studies have been performed on luminous ophiuroids and they have been restricted to two different fields. Ethological approaches on *Ophiopsila californica* have demonstrated that light emission is used as an anti-predatory signal (BASCH, 1988; GROBER, 1988). Physiological works performed on the small brittlestar *Amphipholis squamata* have shown that emitted light is intracellular and restricted to specialized cells called photocytes (DEHEYN et al., 1996). Moreover, this photogenesis is under complex nervous control (DE BREMAEKER et al., 1996, 2000).

Recent works show that natural bioluminescence is an efficient genetic marker in *A. squamata*. Several parame-

Corresponding author: S. Dupont, e-mail: dupont@bani.ucl.ac.be

ters of the bioluminescence (intensity and kinetics), as revealed by KCl stimulation, present important intraspecific variations (DEHEYN et al., 1997; DUPONT & MALLEFET, 2000), which are heritable (DUPONT et al., 2000a). Moreover, bioluminescence reveals exactly the same variability as that observed with molecular markers such as RAPDs (DUPONT et al., 2000b). Natural bioluminescence, polymorphic and heritable, is then a good genetic marker and presents numerous advantages over molecular markers: it is cheaper, faster and easier to use.

The aim of this work was to test this method on other bioluminescent ophiuroid species. We compared the luminous capabilities of individuals of six species using the KCl stimulation method in order to demonstrate that natural bioluminescence can be used as a genetic marker.

MATERIAL AND METHODS

Six species of luminous ophiuroids were collected in different locations (Table 1). Animals were anaesthetised by immersion in 3.5% w/w $MgCl_2$ in artificial sea water. Arms were removed from the disc, measured and stimulated with KCl 200mM to trigger the maximum light emission. Measurements of light capabilities were carried out in a dark room using a luminometer (Berthold FB 12); three parameters were measured to characterize the light response as described for *A. squamata* (MALLEFET et al., 1992). The maximum intensity of light was expressed in megaquanta per seconds and per millimeter of arm (Lmax in $Mq.s^{-1}.mm^{-1}$) and two kinetic parameters were expressed in seconds: the time elapsing between the application of the KCl stimulus and the beginning of the light production (Latency time, Lt), and the time between the beginning of the light production and the maximum of light production (Tlmax). Data were considered as coordinates using the three luminous parameters. Euclidian distances were computed and observations were hierarchically clustered using Ward's maximum-variance method (WARD, 1963). Each mean value is expressed with its standard error of mean (mean ± SEM); analysis of variance (ANOVA) and t-tests were used to determine the significance of the observed differences between the groups. All statistical methods used are designed under the assumption that the data are normally distributed. Tests have been used to check that the data are a random sample from a normal distribution. Since the sample size was less than or equal to 2000, the SHAPIRO-WILK (1965) statistic, W, was used. When data were not normally distributed or when heteroscedasticity occurred, a logarithmic transformation of data was performed as indicated by SOKAL and ROHLF (1995). Analyses were performed using Statistical Analysis System (SAS institute).

TABLE 1

Luminous species of ophiuroids used in this study

	Sampling site
AMPHIURIDAE	
Amphipholis squamata Delle Chiaje, 1828	Tindari, Italy (38°08'N 15°03'E)
Amphiura filiformis Müller, 1776	English Channel, Belgium (54°N 8°E) Fiskebäckskil, Sweden (58°16'N 11°26'E)
Amphiura arcystata Clarck, 1911	Santa Barbara, USA (34°25'N 119°57'W)
Amphiodia n. sp.	Santa Barbara, USA (34°24'N 119°49'W)
OPHIOCOMIDAE	
Ophiopsila aranea Forbes, 1843	Banyuls-sur-Mer, France (42°29'N 3°08'E)
Ophiopsila californica Clarck, 1921	Santa Barbara, USA (34°25'N 119°57'W)

RESULTS

Qualitative description of light emission

In the studied species, bioluminescence is represented by a diversity of colours, localizations and patterns. Two different colours are observed: *Amphiura filiformis* is the only species producing blue luminescence while other species emit in the green. Bioluminescence is always restricted to the arms except in the undescribed *Amphiodia* species where a weak light is also observed in the disc. Representative patterns of light emission of each species are presented in Fig. 1. Luminous reaction presents rapid kinetics in nearly all the species (Table 2): the latency time is short (Lt<2 s) and the maximum intensity of light is quickly reached (Tlmax< 25 s). A contrasting kinetic is observed for *O. aranea* where the luminous reaction is significantly slower (Tlmax>40 s). On average, maximum intensity of light is significantly different between the species ($p<0.01$ except between *A. arcystata – A. squamata* and *O. aranea - O. californica*). *Amphiodia* always produces intense light (30713±2564 $Mq.s^{-1}$.mm-1), followed in level of intensity by both *Ophiopsila* species and *A. filiformis*. *A. squamata* and *A. arcystata* produce a much weaker light, at least ten times less intense than the other species. In addition, there is important intraspecific variability between the three species: *A. filiformis, A. squamata* and *O. aranea*.

Quantitative description of light emission

Inter- and intraspecific differences were formalised by cluster analysis (Ward's methods). As a consequence of the huge variability within the species, *A. filifomis* was analysed separately. Fig. 2 presents the tree inferred from euclidian distances calculated on the basis of the three luminous parameters for *O. aranea, O. californica, A. arcystata, Amphiodia* n. sp. and *A. squamata*, where three different colour morphs were observed on the basis of the

TABLE 2

Luminous parameters for each species (*Amfiura filiformis*, *A. arcystata*, *Amphipholis squamata, Amphiodia n. sp., Ophiopsila aranea* and *O. californica*), colour morphs of *A. squamata* and main clusters for *A. filiformis* (Fig.4) and *O. aranea* (Fig.2). Mean ± standard error of mean; n=number of ophiuroids.

		Lmax ($Mq.s^{-1}.mm^{-1}$)	Lt (s)	Tlmax (s)	n
Ophiopsila aranea	**Mean**	**14679.85±5848.51**	**2.94±1.24**	**51.70±4.82**	**18**
"	Cluster 1 (Fig.2)	3826.52±1195.6	4.16±1.77	43.49±5.08	12
"	Cluster 2 (Fig.2)	36386.52±14186.21	0.48±0.11	68.12±6.50	6
Ophiopsila californica	**Mean**	**19803.89±8230.06**	**0.50±0.02**	**1.88±0.65**	**10**
Amphiura arcystata	**Mean**	**323.10±160.33**	**0.78±0.10**	**9.89±1.19**	**9**
Amphiura filiformis	**Mean**	**8055.39±1270.11**	**0.51±0.04**	**12.69±1.36**	**59**
"	Cluster 1 (Fig.4)	9616.45±2678.60	0.44±0.03	4.95±0.38	12
"	Cluster 2 (Fig.4)	5923.98±4925.69	1.06±0.18	2.38±0.40	4
"	Cluster 3 (Fig.4)	26929.12±5742.82	0.35±0.06	1.16±0.20	6
"	Cluster 4 (Fig.4)	4834.97±724.56	0.35±0.03	24.36±2.20	17
"	Cluster 5 (Fig.4)	7954.76±934.70	0.20±0.00	8.81±1.48	7
"	Cluster 6 (Fig.4)	2824.78±1593.83	0.83±0.08	15.15±1.74	13
Amphipholis squamata	**Mean**	**637.43±153.47**	**1.18±0.09**	**5.84±0.43**	**35**
"	Orange	1.17±0.18	1.62±0.15	4.04±0.29	11
"	Dark-brown	177.25±49.55	1.20±0.11	5.05±0.63	13
"	Spotted	1817.51±215.76	0.71±0.06	8.58±0.46	11
Amphiodia n. sp.	**Mean**	**30713.70±2563.66**	**0.67±0.05**	**3.52±0.41**	**26**

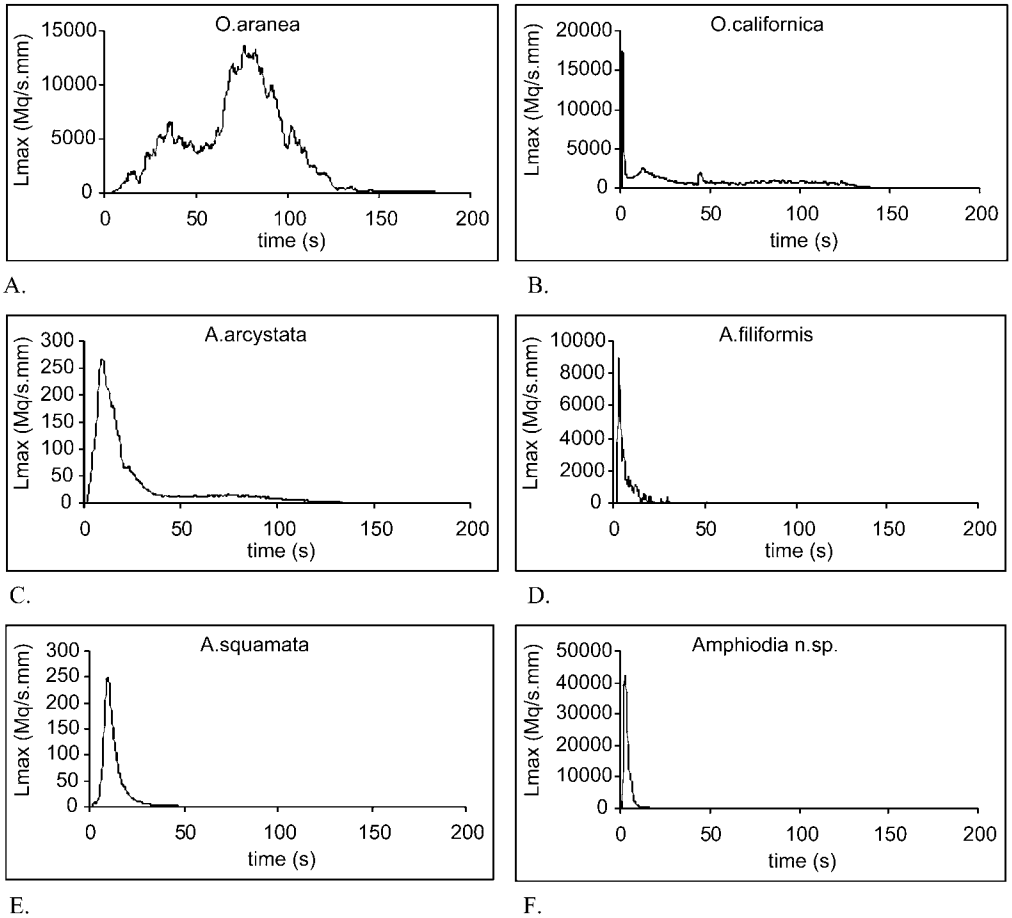


Fig. 1. – Representative recordings of light emitted by an arm stimulated by KCl 200mM (A, *Ophiopsila aranea;* B, *O. californica*; C, *Amphiura arcystata;* D, *A. filiformis;* E, *Amphipholis squamata;* F, *Amphiodia* n.sp.).

pigmentation of arms and discs: Orange, Dark-brown and Spotted (see DUPONT & MALLEFET, 2000). Ward's clustering method revealed six clusters separated by minimal distance of 0.01. Clusters 1 and 2 correspond to *O. aranea*. Cluster 3 contains individuals of *A. arcystata* and *A. squamata* of Dark-brown and Spotted morphs where Orange one constitutes the cluster 4. These four clusters are greatly separated from clusters 5 and 6 containing respectively *Amphiodia* n. sp. and *O. californica*.

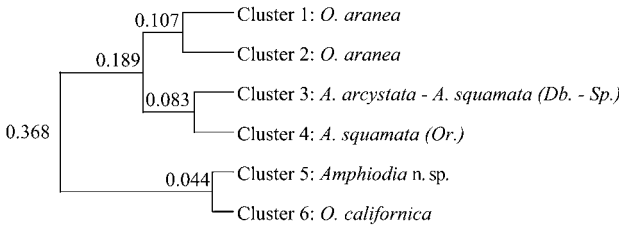
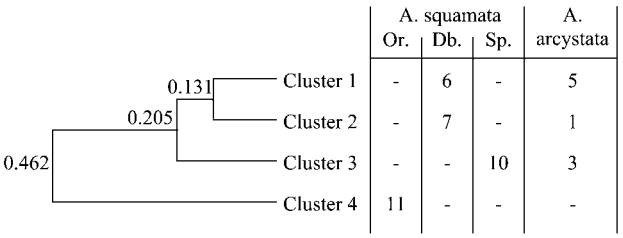


Fig. 2. – Tree inferred from euclidian distances between bioluminescence parameters of *Ophiopsila aranea, O. californica, Amphiodia* n. sp.*, Amphiura arcystata, A. filiformis* and the three colour morphs of *Amphipholis squamata* (Or, orange, Db, dark-brown and Sp., spotted). Distances within a cluster are inferior to 0.01.

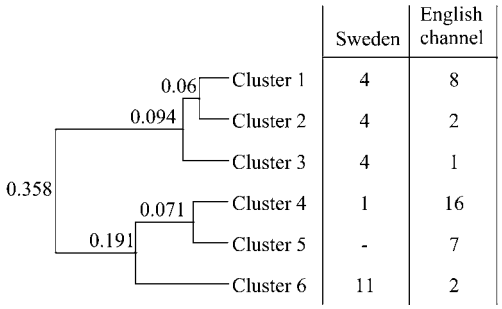
Since it was impossible to isolate *A. arcystata* from *A. squamata,* the same analysis was performed on these species only (Fig. 3). Ward's method then reveals four clusters and allowed the separation of the three colour morphs of *A. squamata* (clusters 1 and 2 are the individuals of the Dark-brown morph; cluster 3, individuals of the Spotted morph and cluster 4, individuals of the Orange ones). Nevertheless, we are unable to isolate *A. arcystata* from Dark-brown and Spotted morphs of *A. squamata* (clusters 1 to 3).



	A. squamata			A. arcystata
	Or.	Db.	Sp.	
Cluster 1	-	6	-	5
Cluster 2	-	7	-	1
Cluster 3	-	-	10	3
Cluster 4	11	-	-	-

Fig. 3. – Tree inferred from euclidian distances between bioluminescence parameters of *Amphiura filiformis* and the three colour morphs of *Amphipholis squamata* (Or, orange, Db, dark-brown and Sp, spotted) and the quantity of ophiuroids of both species in each cluster. Distances within a cluster are inferior to 0.01.

A. filiformis from two different locations (Sweden and the English channel) were analysed with the same methods (Fig. 4). Two group of three clusters (1 to 3 and 4 to 6) are separated by an important distance. This great intraspecific variability cannot be explained by the geographical distribution since all the clusters (except cluster 5) are consist of individuals from the both locations.



	Sweden	English channel
Cluster 1	4	8
Cluster 2	4	2
Cluster 3	4	1
Cluster 4	1	16
Cluster 5	-	7
Cluster 6	11	2

Fig. 4. – Tree inferred from euclidian distances between bioluminescence parameters of *Amphiura filiformis* from two different location (Sweden and English Channel) and the quantity of ophiuroids from each location in each cluster. Distances within a cluster are inferior to 0.01.

DISCUSSION

Looking at bioluminescence, we can see that each species possesses its own particularities: *A. filiformis* is the only one to emit blue light, only *Amphiodia* is able to produce light with its disc, *O. aranea* has a very slow kinetic of light emission, etc. Luminescence is induced by KCl depolarization and characterized by three parameters (Lmax, Lt and Tlmax). Ward's clustering method on euclidian matrix of distance computed on these parameters constitutes an excellent tool giving functional information at inter- and intraspecific levels.

Interspecific variability

Most of the species were isolated using their bioluminescence capabilities. Surprisingly, great differences were observed compared to what was expected with classical phylogeny (SMITH et al., 1995). For example, both species of the genus Ophiopsila, *O. aranea* and *O. californica,* are not closely related. *O. aranea* is clustered with *A. arcystata* and *A. squamata* while *O. californica* is linked to *Amphiodia* n. sp..

These paradoxical results could be due to differences in the nervous control of the photogenesis. Previous works have shown that calcium ions are required to trigger light emission in ophiuroids (MALLEFET et al., 1994, 1998; DEWAEL & MALLEFET, 2000). Nevertheless, the type of calcium channel involved in the luminous control differs from one species to another: L-type channels are involved in the luminous control of *O. californica* whereas another uncharacterized channel type would be implicated in *O. aranea* (DEWAEL & MALLEFET, 2000).

Therefore, we can postulate that our method is useful to reveal physiological differences between species. A comparative study of the nervous control of these species is in progress in order to confirm this hypothesis.

Intraspecific variability

The same method, combining bioluminescence and cluster analysis, was used to reveal variability at the species level. Two species present important intraspecific variability: *A. filiformis* and *A. squamata*.

In *A. squamata*, this variability reflects the polychromatism. Each colour morph is isolated according to its luminous capabilities. This result confirms observations in several populations around the world (Deheyn et al., 1997, 2000; Dupont & Mallefet 2000; Dupont et al., 2000b). Moreover, genetic variations revealed by RAPDs demonstrate that genetic structure is homogenous within each colour morph of a same population (Dupont et al., 2000b). Since there is a link between polychromatism, bioluminescence and genetics, it was suggested that polychromatism and/or bioluminescence might be good indicators of genotype variability.

We propose that this idea could be extended to other ophiuroid species. In the case of *A. filiformis*, the two studied populations (English Channel and Sweden) were not differentiated with the method employed. Moreover, this variability could not be explained by any morphological character such as polychromatism. Assuming that bioluminescence is an indicator of genetic variability in *A. filiformis*, we postulate that most of the genetic variation occurs within population. Similar conclusions were reached by McCormack et al. (2000). They used RAPDs analysis applied to individuals of *A. filiformis* from two geographical locations. Analysis of molecular variance showed that a minimum of 93% of phenotypic variance occurred among individuals within populations.

Conclusion

We propose a new method, using natural bioluminescence properties and cluster analysis, to measure variability within or between species. At the interspecific level, it might give information about differences in the nervous control of luminescence. At the intraspecific level, it could be used as an easy-to-use genetic marker providing information for population genetics of luminous ophiuroids.

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Occurrence of a continental slope decapod crustacean community along the edge of the minimum oxygen zone in the south eastern Gulf of California, Mexico

Michel E. Hendrickx

Instituto de Ciencias del Mar y Limnología, Unidad Académica Mazatlán
P.O. Box 811. Mazatlán, Sinaloa, 82000, Mexico

ABSTRACT. Decapod crustaceans living in deep-water in the south eastern Gulf of California were collected during two research cruises in August 1991 and 2000. Benthic sledges were operated in the depth range of 550 to 2250 m. Vertical distribution of dissolved oxygen was obtained at selected stations and epibenthic oxygen content was measured at almost all sampling stations. A total of 31 species was collected, 21 strictly benthic and eight strictly pelagic. They belong to the Penaeoidea (five species), the Caridea (13 species), the Anomura (seven species), the Astacidea (one species), the Thalassinoidea (two species), the Eryonoidea (two species) and the Brachyura (one species). The oxygen minimum zone at bottom level represents a dispersal barrier for continental shelf species, including species known to have a wide bathymetric distribution. The deep-water decapod crustacean fauna (i.e., below 550 m) living along the offshore edge of this oxygen minimum zone is dominated by species of *Munidopsis*. The number of benthic species collected at the stations varied considerably, from zero to 15. The highest numbers of species were caught in the depth range of 1188-1245, where hypoxic (0.6-0.76 ml O2/l) conditions prevailed. Stations with higher oxygen content had fewer benthic species. Factors other than oxygen content, affect the occurrence of species in the area.

KEY WORDS: Decapod crustaceans, continental slope, southeastern Gulf of California, hypoxia.

INTRODUCTION

While the northern part of the Gulf of California is relatively shallow, with the exception of the Delfin basin, the central and southern parts increase in average depth towards the mouth of the Gulf. Several deep basins are found offshore with depths reaching 3000 m or more. The southern Gulf of California opens to the Pacific, with depths greater than 3000 m at the entrance (PARKER, 1964; ALVAREZ-BORREGO & SCHWARTZLOSE, 1979). In the southern Gulf, water temperature does not show any special structure. It decreases monotonically with depth and reaches values of less than 10 °C below 400-500 m. There is an orderly progression to the deepest portions of the Gulf of from 10 °C to <4 °C at 2000 m. The southernmost basin, from about 26º N to the mouth of the Gulf, has epibenthic water temperatures of 2 °C or less, characteristic of the bottom water of the equatorial Pacific (PARKER, 1964).

Epibenthic dissolved oxygen concentration has been recognized as a major limiting factor for benthic and demersal species. Although it is generally admitted that respiration in most marine invertebrates is not significantly affected until extremely low oxygen concentrations are reached, i.e. below 2.0 ml/l or even less (ROSENBERG et al. 1991; DIAZ & ROSENBERG, 1995), decreasing oxygen content near the bottom or in the water column can create an anoxic zone where no macrofauna occur (DIAZ & ROSENBERG, 1995). Species diversity at depths from 100 m to 2140 m off the coast of Southwest Africa, and at depths of 1100-1300 m in the Santa Catalina Basin, off California, has been shown to decline with reduced oxygen content of the bottom water or to be lower than adjacent areas where oxygen content is higher (JUMARS, 1976; GRASSLE, 1989).

Oxygen minimum zones have long been recognized in different parts of the world, either in fjords, coastal water

Corresponding author: M. Hendrickx, e-mail: michel@mar.icmyl.unam.mx

or in open ocean. The widest area where severe hypoxia has been observed is in the east Pacific (WYRTKI, 1966; KAMYKOWSKI & ZENTARA, 1990). Severe hypoxia can drastically reduce diversity and size of natural communities (see NILSSON & ROSENBERG, 1994).

Since deep water crustaceans were collected by the "Albatross" in 1891 off the coast of Mexico to Peru, almost no studies have been performed in the southern Gulf of California. The most complete study of the deep water fauna of the east Pacific slope is by PARKER (1964), based on series of 11 samples obtained on the middle continental slope (731-1799 m), and of 19 samples obtained between 1800 and 4122 m in the abyssal southern basins and outer slope of the Gulf of California. Parker's definition of assemblages, however, was based mostly on molluscs and the occurrence of decapod crustaceans must be inferred from a long data matrix (Parker, 1964: Table 7) and related to a map of sampling stations. He found 14 species of decapod crustaceans below 500 m of which only two were recorded in the Southeastern Gulf of California. WICKSTEN (1989) provided a synthesis of all species of eastern Pacific decapod crustaceans with the majority of records at 50 m or deeper (defined as "offshore species"). A total of 183 species is included, of which 117 have records in the depth category of 500-1000 m, 96 at 1000-1500 m and 46 at depths >1500 m (WICKSTEN, 1989). An analysis of habitats and biodiversity of decapod crustaceans in the Southeastern Gulf of California (HENDRICKX, 1996a) reported that 19 deepwater species were collected between 200 and 1200 m, some of which represent new records for the area (HENDRICKX, 1996b).

An oxygen minimum zone has long been recognized in the Gulf of California. According to PARKER (1964), this zone of low oxygen concentration (ca. 0.5 ml/l) at or near the bottom forms a fringe parallel to the coast that extends, on both sides of the Gulf, from the east and west entrances to ca. 29° N. Up to 90 km wide, it mostly covers the outer continental shelf and the upper slope, roughly from 100m to 500 or 1000 m, depending on location. Recent studies, however, indicate that almost anoxic conditions are occasionally found on the shelf at 60 m (HENDRICKX et al., 1984; GARDUÑO-ARGUETA & CALDERÓN PÉREZ, 1995).

The purpose of this study is to define the deepwater decapod crustacean community that occurs on the offshore side on the oxygen minimum, roughly in the depth range of 550 to 2200 m, in the SE Gulf of California.

METHODS

Crustaceans samples were dredged at depths of 550 to 2250 m in the SE Gulf of California. Material was obtained during two cruises aboard the R/V "El Puma" of the Universidad Nacional Autónoma de Mexico. Samples were obtained with benthic sledges at 11 stations in August 1991 (TALUD III cruise) and at eight stations in August 2000 (TALUD IV), off the coast of Sinaloa, Mexico (Table 1). The sledges were operated at depths from 550 to 1380 m (TALUD III) and from 785 m to 2250 m (TALUD IV). Two different benthic sledges were used: a 2.5 m wide by 1.0 m high modified Agassiz sledge equipped with a collecting net of ca. 5.5 cm (2 1/4") stretch mesh lined with ca. 2.0 cm (3/4") mesh net (TALUD III) and a 2.35 m wide by 0.95 m high standard benthic sledge equipped with a collecting net of ca. 5.5 cm (2 1/4") stretch mesh lined with ca. 2.0 cm (3/4") mesh net in the mouth area (TALUD IV). At selected sampling stations of the TALUD III cruise and at all sampling stations of TALUD IV cruise, a previously calibrated CTD probe equipped with an oxygen sensor was used to obtain temperature, salinity and dissolved oxygen profiles from surface to near bottom level. During the TALUD IV cruise, an opening-closing bottle was used to obtain near bottom water samples used to measure the dissolved oxygen by the Winkler method (duplicate samples). During the TALUD III cruise, oxygen measurements were obtained from the pre-calibrated probe (a SEB Seacat Profiler) and are considered reliable for biological interpretation. The CTD probes and opening-closing bottle were not operated less than 15 m off the bottom.

TABLE 1

Sampling stations where decapod crustaceans were caught during the TALUD III and IV cruises.

Station	Date	Position	
		Lat. N	Long. W
10/III	18/Aug/1991	23.41.9	107.31.8
10A/III	18/Aug/1991	23.44.3	107.38.6
14A/III	19/Aug/1991	24.38.8	108.26.9
14B/III	19/Aug/1991	24.39.2	108.37.8
19/III	20/Aug/1991	25.12	109.07
20A/III	24/Aug/1991	25.12.6	109,06
24/III	21/Aug/1991	25.33.6	109.42.02
24A/III	24/Aug/1991	25.45.2	109.46.8
4/IV	23/Aug/2000	21.59.0	106.35.0
13/IV	24/Aug/2000	23.17.51	107.29.85
14/IV	24/Aug/2000	23.13.4	107.41.8
18/IV	25/Aug/2000	24.15.2	108.17.1
19/IV	25/Aug/2000	24.15.3	108.24.1
20/IV	25/Aug/2000	24.27.4	108.35.26
25/IV	26/Aug/2000	24.53.2	108.59.4
26/IV	26/Aug/2000	24.56.4	109.05.6
27/IV	26/Aug/2000	24.59.0	109.12.1
33/IV	27/Aug/2000	25.45.9	109.48.1
34/IV	27/Aug/2000	25.40.67	109.54.4
35/IV	27/Aug/2000	25.53.98	110.11.29

Crustaceans were sorted onboard, preserved in diluted formaldehyde or in 70% ethanol. A list of all species is tabulated with accompanying data. Body lengths were measured with vernier calipers, usually to the nearest

0.1 mm. Carapace length was measured in shrimps while total length was used with lobsters and galatheids. The identified specimens form part of the collections of the Mazatlán Marine Station, UNAM.

Only species collected during the TALUD IV cruise and of special interest were treated individually in the systematic section. Biogeographic or ecological data related to species collected during the TALUD III cruise have been reported elsewhere (see HENDRICKX, 1996b). Data on other species captured during these cruises are summarized and included in a general table with their respective collecting data.

Abbreviations used: St., sampling station; CL, carapace length; TL, total length; BS, benthic sledge.

RESULTS

Systematic section

PENAEOIDEA

Family Solenoceridae

***Hymenopenaeus doris* (Faxon, 1893)**

Haliporus doris FAXON, 1893: 214.

Haliporus doris. – FAXON, 1895: 191, pl. 49, Figs. 1-1c.

Hymenopenaeus doris. – BURKENROAD, 1936: 104; 1938: 60. – PÉREZ-FARFANTE, 1977: 283, Figs. 9, 17a, 18a, 19-20. – MÉNDEZ, 1981: 55, Figs. 155, 156, 156a-c. – HANAMURA, 1983: 55, Fig. 2. – WICKSTEN, 1989: 311. – WICKSTEN & HENDRICKX, 1992: 4. – HENDRICKX, 1993: 305; 1995d: 529, Fig. 1b, 530, Fig. 3b, 531, Fig. 8a, 532, Fig. 9a, 534; 1996c: 119, Fig. 60. – HENDRICKX & ESTRADA-NAVARRETE, 1996: 40, Fig. 24.

Aliporus doris. – DEL SOLAR, 1972: 4.

Material examined. – St. 19-IV, 25/VIII/2000, 1 male (CL 13.5 mm) and 1 female (CL 12.0 mm), 1245-1240 m, BS.

Previously known distribution. – From Punta Chivato, Mexico to Guanape, Peru (HENDRICKX, 1996c).

Remarks. – The only records in benthic samples for the Gulf of California are off Punta Arena and Punta Chivato, along the Baja California Peninsula (HENDRICKX, 1996c). The species has also been captured in the water column (see HENDRICKX & ESTRADA-NAVARRETE, 1996) and its presence in bottom trawls might be due to incidental catch during recovery of the gear.

Family Benthesicymidae

***Benthesicymus tanneri* Faxon, 1893**

Benthesicymus tanneri FAXON, 1893: 215.

Benthesicymus tanneri. – FAXON, 1985: 205, Fig. H. – RATHBUN, 1904: 147. – SCHMITT, 1921: 23, Fig. 10. – MÉNDEZ, 1981: 31. – RODRÍGUEZ DE LA CRUZ, 1987: 20. – WICKSTEN & HENDRICKX, 1992: 2. – HENDRICKX, 1993: 305; 1995d: 436, Fig. 4, 437; 1996c: 12, Fig. 5.

Material examined. – St. 13-IV, 24/VIII/2000, 1 male (CL 31.8 mm), 1530-1520 m, BS; St. 19-IV, 25/VIII/2000, 3 females (CL 36.5-37.3 mm), 1245-1240 m, BS; St. 26-IV, 26/VIII/2000, 1 male (CL 30.2 mm) and 2 juveniles (not measured), 1225-1240 m, BS; St. 33-IV, 27/VIII/2000, 2 males (CL 33.2 and 34.8 mm), 1040 m, BS.

Previously known distribution. – From San Diego, California, USA to Ilo, Peru. Southeastern Gulf of California; Galapagos Islands (HENDRICKX, 1996c).

Remarks. – The distribution of this species is synthesized by HENDRICKX (1996c). *Benthesicymus tanneri* is a common species in deep water of the SE Gulf of California. Material captured during this study includes the largest specimens known to date (up to 135 mm TL). Adult specimens are rather heavy (ca. 13.0-16.5 g of individual fresh weight for specimens of 110-135 mm TL) and they most probably live close to the bottom. Often captured with *Heterocarpus affinis*, a potential deep-water resource in the eastern tropical Pacific (HENDRICKX 1995d), *B. tanneri* could represent an interesting by-catch species in pandalids fishery.

CARIDEA

Family Pandalidae

***Pandalus amplus* (Bate, 1886)**

Pandalopsis amplus BATE, 1888: 671, pl. 175, Fig. 3.

Pandalopsis ampla. – FAXON, 1895: 155. – RATHBUN, 1904: 51. – SCHMITT, 1921: 46. – WICKSTEN, 1989: 313. – WICKSTEN & HENDRICKX, 1992: 9.

Material examined. – St. 19-IV, 25/VIII/2000, 2 females, the smallest ovigerous (CL 31.6 and 32.6 mm), 1245-1240 m, BS.

Previously known distribution. – From Sea Lion Rock, Washington, USA to Acapulco, Gro., Mexico. ATL from off Montevideo, Uruguay to Argentina (WICKSTEN & HENDRICKX, 1992).

Remarks. – *Pandalus amplus* is a common species off the coast of California, where it has been reported at least in 28 localities (HENDRICKX & WICKSTEN, 1989) and from a depth range of 132-2000 m. There are only five records along the Pacific coast of Mexico, including two in the Gulf of California. The present record represents a range extension of this species along the east coast of the Gulf of California to 24°15.3' N - 108°24.1'W. The size of the largest female (165 mm TL) is close to the maximum known size (170 mm TL) for the east Pacific. Fresh weight of specimens examined are 22 and 24 g.

Family Crangonidae

***Sclerocrangon atrox* Faxon, 1893**

Sclerocrangon atrox Faxon, 1893: 199.

Sclerocrangon atrox. – FAXON, 1895: pl. 35, Figs. 1, 1a-f. – MÉNDEZ, 1981: 121. – WICKSTEN & HENDRICKX, 1992: 6.

Material examined. – St. 13-IV, 24/VIII/2000, 1 male (CL 27.8 mm), 1530-1520 m, BS; St. 19-IV, 25/VIII/2000, 1 male (CL 25.2 mm), 1245-1240 m, BS.

Previously known distribution. – From near Tres Marias Islands, Nayarit, Mexico to off Mollendo, Peru (WICKSTEN & HENDRICKX, 1992).

Remarks. – A large (up to 162 mm TL; ca. 38 mm CL) and rather rare species of Crangonidae. The present material increases the range northwards by two degrees of latitude, from off Tres Marias Islands to 24º15.3'N - 108º24.1'W.

Family Oplophoridae

***Acanthephyra brevirostris* Smith, 1885**

Acanthephyra brevirostris Smith, 1885: 504.

Acanthephyra brevirostris. – Smith, 1886(1887): 670, pl. 14, Fig. 2, pl. 15, Figs. 2, 8, pl. 16, Figs. 1, 6. – Faxon, 1895: 167. – Kensley, 1972: 38, Fig. 17m. – Crosnier & Forest, 1973: 41, Figs. 8c-d. – Hanamura, 1983: 75. – Chace, 1986: 8 (Key), Figs. 2e, 4e, 5e, 6d, 8d. – Hendrickx & Estrada-Navarrete, 1989: 113; 1996: 109, Fig. 67.

Material examined. – St. 26-IV, 26/VIII/2000, 1 juvenile (CL 6.5 mm), 1225-1240 m, BS.

Previously known distribution. – From off Baja California and off Ecuador, including the SE Gulf of California; SW Indian Ocean; East and West Atlantic Ocean (Hendrickx & Estrada-Navarrete, 1996).

Remarks. – Only two records of this species are available for the east Pacific (off Ecuador, 0.58' S - 115.15' E; Dowd Tablemount, off Baja California, Mexico) (Hendrickx & Estrada Navarrete, 1996). The juvenile examined, although slightly damaged, shows features distinctive of this species. This deep-water pelagic species has been collected between 1280 and 5394 m, mostly in mid-water trawls. Examined material was most probably collected in the water column during recovery of the dredge.

***Hymenodora gracilis* Smith, 1887**

Hymenodora gracilis Smith, 1886 (1887): 680, pl. 12, Fig. 6.

Hymenodora gracilis. – Sivertsen & Holthuis, 1956: 16, Figs. 12, 13. – Crosnier & Forest, 1973: 83, Fig. 25a. – Wasmer, 1986: 49, Figs. 10b-c. – Chace, 1986: 43 (clave), Figs. 21p-t. – Iwasaki & Nemoto, 1987a: 20. – Krygier & Wasmer, 1988: 87. – Hanamura, 1989: 54, Fig. 2. – Hendrickx & Estrada-Navarrete, 1989: 115; 1996: 119, Fig. 73. – Allen & Butler, 1994: 426, Fig. 5.

Material examined. – St. 35-IV, 27/VIII/2000, 1 male (CL 12.0 mm), 2016-2020 m, BS.

Previously known distribution. – From Oregon, USA to West coast of Baja California; coast of Chile and in subantarctic waters of the South Pacific Ocean (Hendrickx & Estrada-Navarrete, 1996).

Remarks. – This is the first record outside the temperate waters of the NE and SE Pacific. A mesopelagic and bathypelagic species, *H. gracilis* is reported from ca. 300 to 5300 m (Hendrickx & Estrada Navarrete, 1996). Some of these depth records, however, might be erroneous caused by the use of non-closing, bottom sampling devices. The material reported here was accidentally captured in the bottom sledge and there is no way to assess correctly the depth at which it was collected. *Hymenodora glacialis*, a closely related species, has been previously recorded in the central Gulf of California (Hendrickx & Estrada Navarrete, 1996).

Family Pasiphaeidae

***Pasiphaea emarginata* Rathbun, 1902**

Pasiphaea emarginata Rathbun, 1902: 905.

Pasiphaea emarginata. – Rathbun, 1904: 22, Fig. 4. – Schmitt, 1921: 30, Fig. 15. – Chace, 1937: 110. – Word & Charwat, 1976: 205-206 (Illustration). – Hendrickx & Estrada-Navarrete, 1989: 111; 1996: 89, Fig. 55.

Material examined. – St. 25-IV, 26/VIII/2000, 2 males (CL 35.1 and 36.0 mm) and 10 females (CL 27.3-38.3 mm), 870-835 m, BS.

Previously known distribution. – From West coast of Baja California (up to 26° N) and in the Gulf of California (up to 30°11' N); south to the Gulf of Panama and Lobos de Tierra Islands, Peru; Galapagos Islands (Hendrickx & Estrada-Navarrete, 1996).

Remarks. – In addition to the type material taken near Concepcion Bay entrance, the only other two records of this species in the Gulf of California are over 60 years old (Chace, 1937); all these records are from the central Gulf.

***Pasiphaea magna* Faxon, 1893**

Pasiphaeia magna Faxon, 1893: 209.

Pasiphaea magna. – Faxon, 1895: 176, pl. 45, Fig. 2. – Word & Charwat, 1976: 208. – Méndez, 1981: 64, Figs. 190-192. – Krygier & Wasmer, 1988: 77. – Hendrickx & Estrada-Navarrete, 1989: 111; 1996: 91, Figs. 56, 57.

Material examined. – St. 19-IV, 25/VIII/2000, 1 female (CL 31.1 mm), 1245-1240 m, BS.

Previously known distribution. – From Oregon, USA, and the SE Gulf of California to the Gulf of Panama and Peru (Hendrickx & Estrada-Navarrete, 1996).

Remarks. – This is the second record of *P. magna* in Mexican waters (see Hendrickx & Estrada-Navarrete, 1996). Maximum recorded size is 185 mm (TL). The examined specimen is 106 mm TL.

Family Hippolytidae

***Lebbeus scrippsi* Wicksten & Méndez, 1982**

Lebbeus scrippsi Wicksten & Méndez, 1982: 106.

Lebbeus scrippsi. – Wicksten, 1989: 312. – Hendrickx, 1996b: 946.

Material examined. – St. 19-IV, 25/VIII/2000, 4 males (CL 6.7-8.2 mm) and 3 ovigerous females (CL 9.5-10.9 mm), 1245-1240 m, BS; St. 26-IV, 26/VIII/2000, 2 males (CL 6.7 and 10.3 mm), 1225-1240 m, BS.

Previously known distribution. – From Peru to Chile; one record in southeastern Gulf of California (Wicksten, 1989; Hendrickx, 1996b).

Remarks. – This rare species is again reported in the area; two females (CL 9.0 and 9.4 mm), including one ovigerous, were reported in 1996. Among the 9 specimens reported here are 3 ovigerous females carrying 32-66 eggs each (Table 2). The largest female from station 26 (CL 10.9 mm) is 42.0 mm TL, slightly longer than the largest paratype (TL 41.2 mm).

TABLE 2

Sizes by sex, eggs number and eggs size in *Lebbeus scrippsi* from the Gulf of California continental slope (M, male; F, female).

Sex	CL/TL(mm)	Eggs Number	Size range (mm)
M	6.7/26.8	---	---
M	7.0/30.1	---	---
M	8.2/35.8	---	---
M	8.2/36.9	---	---
F	9.5/32.0	32	1.35-1.65
F	10.0/36.2	35	2.10-2.55
F	10.9/42.0	66	1.58-2.02
M	10.3/36.5	---	---
M	6.75/30.9	---	---

Family Glyphocrangonidae

***Glyphocrangon sicaria* Faxon, 1893**

Glyphocrangon sicarius Faxon, 1893 : 202.

Glyphocrangon sicaria. – Faxon, 1895 : pl. 39, Figs. 1, 1a-e. – Wicksten, 1989 : 314. – Wicksten & Hendrickx, 1992 : 6.

Material examined. – St. 14-IV, 24/VIII/2000, 1 specimen not sexed (CL 6.5 mm), 2160-2150 m, BS; St. 26-IV, 26/VIII/00, 1 specimen not sexed (CL 9.0 mm), 1225-1240 m, BS.

Previously known distribution. – From South of Punta Guiones, Costa Rica to Gulf of Panama (Wicksten & Hendrickx, 1992).

Remarks. – One of the 5 species of the genus reported for the eastern tropical Pacific, *G. sicaria* has so far been reported exclusively below ca. 1454 m (1454-3310 m; Wicksten, 1989).

Family Nematocarcinidae

***Nematocarcinus* cf. *ensifer* (Smith, 1882)**

Eumiersia ensifera Smith, 1882 : 77, pl. 13, Figs. 1-9.

Nematocarcinus ensiferus. – Smith, 1884 : 368, pl. 7, Fig. 1.

Nematocarcinus ensifer. – Crosnier & Forest, 1973 : 116, Figs, 32a-c, 33a-c. – Wicksten & Hendrickx, 1992 : 6.

Material examined. – All specimens unsexed and unmeasured. St. 13-IV, 24/VIII/2000, 6 specimens, 1530-1520 m, BS; St. 26-IV, 26/VIII/2000, 5 specimens, 1225-1240 m, BS; St. 33-IV, 27/VIII/2000, 2 specimens, 1040 m, BS; St. 34-IV, 27/VIII/2000, 1 specimen, 1240-1250 m, BS.

Previously known distribution. – *Nematocarcinus ensifer* is known from off Acapulco, Mexico, to the Galapagos Islands; distributed worldwide (Atlantic, Pacific, Indian Ocean and Mediterranean Sea (Wicksten & Hendrickx, 1992).

Remarks. – The material is assigned to *N. ensiger* with some doubts. It includes specimens with no ventral teeth on the rostrum and others with 1-3 clearly distinguishable small teeth in the distal portion. According to Crosnier and Forest (1973), presence of ventral teeth on some *Albatross* specimens, reported by Faxon (1895) as belonging to an atypical form of "*N. ensifer*" from the east Pacific, should be linked with the presence, in this area, of another species. A revision of W. Faxon material by R. Burukovsky (pers. comm. October 2000) prompted this author to describe a new species (forthcoming manuscript) close to *N. agassizi* Faxon, 1893, the second species of this genus previously reported in the east Pacific. Although our material features a rostrum clearly distinct from *N. agassizi*, a careful revision of the specimens collected during both TALUD cruises (see Hendrickx, 1996b) should be undertaken. Although the dorsal process on the posterior margin of the third abdominal segment is similar in large, adult specimens, the rostrum features either long dorsal spines and a few ventral spines, or short dorsal spines on a longer rostrum and no ventral spines (Fig. 1). As noted by R. Burukovsky, species of *Nematocarcinus* are difficult to identify, mostly because they are generally damaged during sampling (loss of pereiopods).

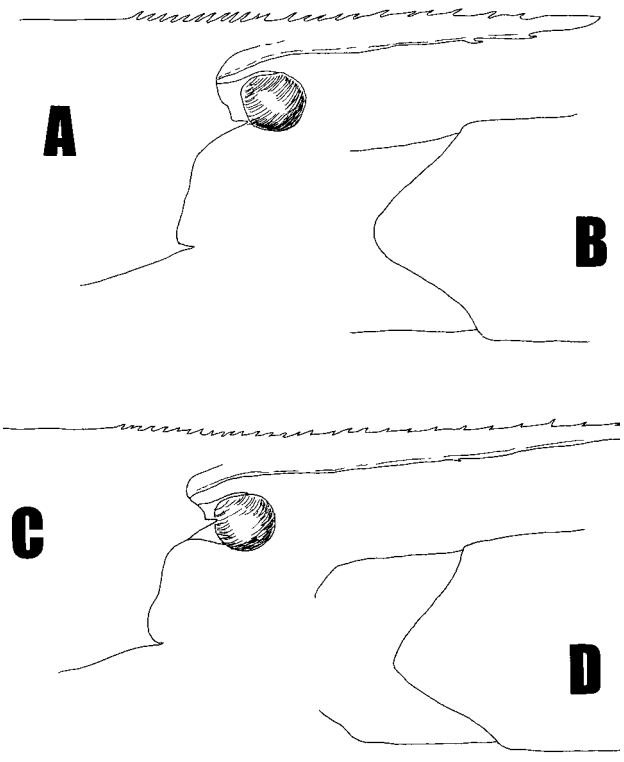

Fig. 1. – A, C) Anterior part of carapace and rostrum of *Nematocarcinus* cf. *ensifer*. B, D) Dorsal view of 3rd abdominal tergite (A, B, St. 34. CL 16.6 mm; C, D, St. 26, CL 22.2 mm).

ERYONOIDEA

Family Polychelidae

***Stereomastis pacificus* (Faxon, 1893)**

Polycheles sculptus pacificus Faxon, 1893 : 196.

Polycheles sculptus pacificus. – Faxon, 1895: 122, pl. C, Fig. 1, 1a.

Eryoniscus agassizi. – Schmitt, 1921 : 105, pl. 15, Figs. 1-2.

Stereomastis sculpta pacifica. – Wicksten, 1981 : 914, Fig. 1 ; 1989 : 311. – Hendrickx, 1995a : 156.

Material examined. – St. 19-IV, 25/VIII/00, 1 male (TL 118.0 mm), 2 females (CL 105.0 and 108.0 mm) and 1 ovigerous female (CL 129.0 mm), 1245-1240 m, BS.

Previously known distribution. – From San Clemente Island, California, USA, to Valparaiso, Chile. Off Tres Marias Islands (Hendrickx, 1995a).

Remarks. – There is a total of 41 records of *S. pacificus* from the east Pacific (off the coasts of southern California, USA, Mexico, Panama, Costa Rica, Colombia, Peru and Chile) (see Wicksten, 1981), thus indicating that it has been frequently caught within its range. Original records by Faxon (1893) include one from the SE Gulf of California, off Tres Marias Islands ; this is believed to be the only published record for this species within the Gulf of California. Wicksten (1981) mentioned a series of 32 adults from 20 stations in Costa Rica, Mexico and southern California, but without further details.

ANOMURA

Family Galatheidae

***Munidopsis ciliata* Wood-Mason, 1891**

Munidopsis ciliata Wood-Mason, 1891 : 200.

Munidopsis ciliata. – Faxon, 1895 : 84, pl. 18, Fig. 3. – Benedict, 1902 : 318. – Ambler, 1980 : 19, Fig. 3. – Wicksten, 1989 : 315. – Hendrickx & Harvey, 1999 : 376.

Munidopsis brevimana. – Henderson, 1885 : 414 ; 1888 : 154, pl. 17, Figs. 1, 2.

Munidopsis (*Orophorhynchus*) *ciliata*. – Alcock, 1901 : 267.

Material examined. – St. 19-IV, 25/VIII/00, 2 males (TL 45.7 and 52.3 mm) and 1 ovigerous female (TL 58.8 mm), 1245-1240 m, BS.

Previously known distribution. – From Oregon, USA to off Panama, including the southern Gulf of California (Wicksten, 1989).

Remarks. – Within its geographic range, *M. ciliata* had been reported from a depth range of 2030-2075 m (Wicksten, 1989). The present record is from much shallower water. The ovigerous female carried 56 eggs.

***Munidopsis depressa* Faxon, 1893**

Munidopsis depressa Faxon, 1893 : 189.

Munidopsis depressa. – Faxon, 1895 : 96, pl. 22, figs 2, 2a, 2b. – Benedict, 1902 : 319. – Wicksten, 1989 : 315. – Hendrickx, 1996b : 946. – Hendrickx & Harvey, 1999 : 376.

Material examined. – St. 25-IV, 26/VIII/2000, 205 males (TL 8.5-37.0 mm), 64 females (TL 12.0-35.0 mm) and 4 ovigerous females (TL 27.0-33.0 mm), 870-835 m, BS ; St. 26-IV, 26/VIII/2000, 33 males (TL 12.5-37.0 mm), 13 females (TL 16.0-35.0 mm) and 3 ovigerous females (TL 27.0-35.0 mm), 1225-1240 m, BS. St. 33-IV, 27/VIII/2000, 1 male (TL 36.0 mm) and 1 ovigerous female (TL 38.0 mm), 1040 m, BS.

Previously known distribution. – From off Santa Catalina Island, California, USA, to the Gulf of California, Mexico, from off Ahome Point, Sinaloa, to off Tres Marias Islands (Hendrickx, 1996b).

Remarks. – Five samples were obtained during the previous cruise (TALUD III) at depths between 820 and 1208 m in the same area (Hendrickx, 1996b). Considering these records and the new material obtained during this study, *M. depressa* appears to be one of the most common and abundant species of decapod crustacean on the continental slope in the SE Gulf of California.

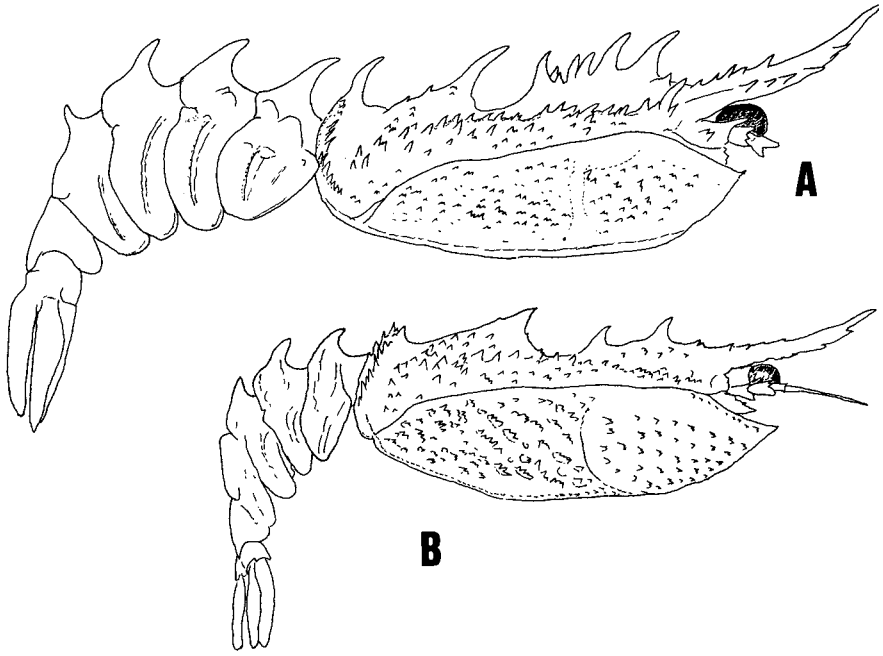

Fig. 2. – Lateral view of *Munidopsis depressa*. A) Specimen with unusually strong and long spines on carapace and a dorsal tooth on 5th abdominal segment (St. 33). B) Type material (after Faxon, 1893).

Specimens from stations 26 and 33 represent a spinose form of *M. depressa*. Spines on the carapace are much stronger and more numerous; also, the dorsal teeth on the abdominal segments are much stronger and there is one dorsal tooth on the fourth segment (Fig. 2), while material from the other stations lack this tooth, as does the type material illustrated by FAXON (1893). Close examination of these spinose specimens, including shape of the third maxilliped, the basal article of the antenna and antennula, the sternum and the chelipeds, indicates that this spinose form corresponds to an extreme variation of *M. depressa*.

Of the total (324 specimens), 239 specimens were males (74%). Only 8 ovigerous females were collected. Number of eggs varied from 21 to 29 in the smallest females (i.e., TL 27.0 to 33.0 mm) to 68 in the larger specimen collected at station 33; three hatching females had only 7 eggs retained on the pleopods.

Munidopsis palmatus Khodkina, 1973

Munidopsis palmatus KHODKINA, 1973:1164-116, Figs 5-6.

Material examined. – St. 19-IV, 25/VIII/00, 1 ovigerous female (TL 22.0 mm), 1245-1240 m, BS; St. 26-IV, 26/VIII/2000, 1 ovigerous female (TL 24.0 mm),1225-1240 m, BS.

Previously known distribution. – Known only from the type locality, off the coast of Chile, north of Valparaiso (32º11'6" S – 71º46'3" W).

Characteristics. – Rostrum triangular in dorsal view, about 1/3 carapace length. Carapace as long as wide; gastric and cardiac regions raised, strong antero-branchial protuberance, granulated, tipped with spine or blunt tubercle. Antennal spine blunt or obsolete; protuberance at antero-lateral angle tipped with strong tooth, curved inside; lateral margin posterior to cervical groove entire, granulated. Dorsal carapace roughly granulated; cervical groove poorly marked, pair of large, strong gastric tubercles and conical median tubercle beyond this pair; posterior margin raised,.

Surface of sternite 4-6 smooth; sternites 5 to 7 with transverse ridges minutely granulate, not raised. Abdominal segments without spines or tubercles, punctate; transverse ridge on segments 2-4 strong.

Eyes small, without pigmentation and without spines, diameter less than 1/2 length of rostrum.

Antennular basal segment broad, bearing large ventro-internal spine with accessory spinules, sharp external spine and two distal spines, dorsal one directed upwards; outer margin granulated. Outer distal spine of basal antennal peduncle strong, sharp, not reaching distal margin of 3rd segment of antennular peduncle. Merus of third maxiliped with two strong spines on flexor margin (no distal spine); extensor margin armed with one distal spine.

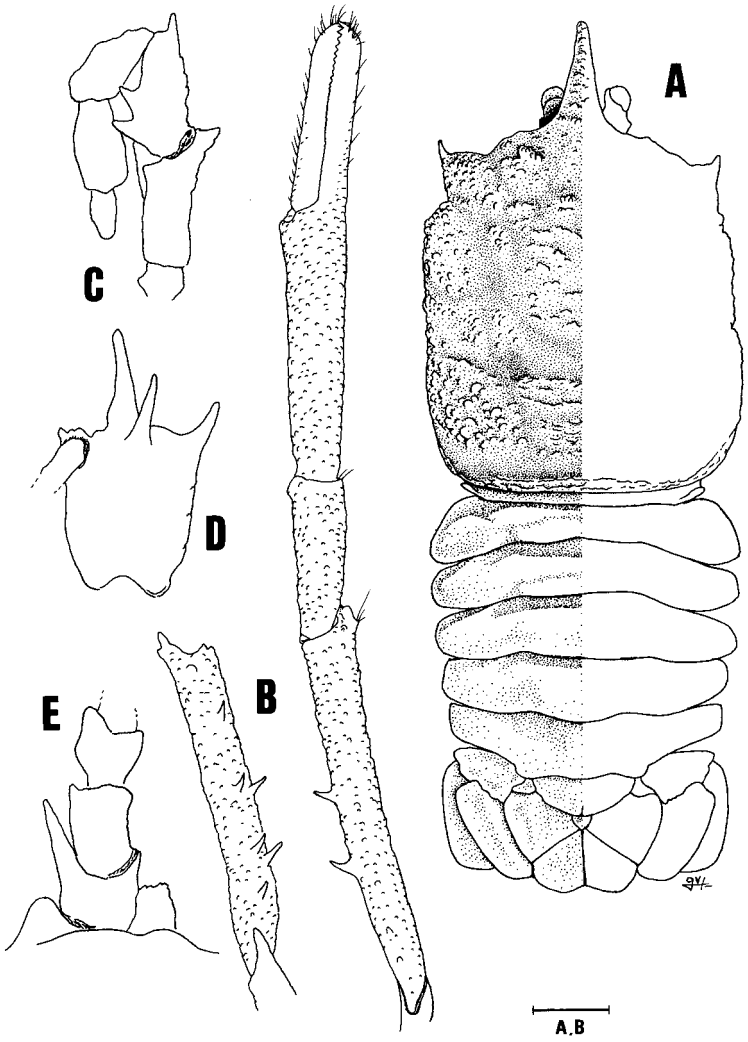

Fig. 3. – *Munidopsis palmatus*. A, Dorsal view. B) Left cheliped, dorsal view, and ventral view of merus. C) Left third maxilliped, lateral view. D) Right basal segment of antennula, dorsal view. E, Left antenna basal segments, dorsal view (scale = 2 mm).

Cheliped about three times as long as carapace, granulated; merus with 5-6 sharp spines on inner margin, one distal. Carpus without spine; chela without spines, longer than merus, flat; palm slightly longer than fingers, wider distally.

Lower and upper margin of manus spineless; fingers flattened, ventral margin of fixed finger and dorsal margin of dactylus spineless. Carpus subcylindrical, about 2/3 length of palm; dactylus shorther than palm.

Pereiopods slender, without spines. Propodus about 3/4 merus length; dactylus falciform, more than half propodus length.

Remarks. – This species, together with three other species described from off the coast of Chile by KHODKINA (1973: *M. verrucosus*, *M. cochlearis* and *M. follirostris*) was overlooked by RETAMAL (1981) and WICKSTEN (1989) in their respective review of the decapod crustaceans of Chile and analysis of the deep-water decapod crustaceans fauna of the east Pacific. The rediscovery of *M. palmatus*, previously known only from the male holotype (8.9 mm CL), in the SE Gulf of California indicates that this species is probably distributed throughout the east Pacific. The original description was in Russian, so the characteristics distinguishing this species from other *Munidopsis* present in the area are presented above.

Both specimens were ovigerous females. The specimen from station 19 carried 7 eggs but had obviously started hatching prior to capture; the female from station 26 carried 12 eggs.

Munidopsis quadrata Faxon, 1893

Munidopsis quadrata FAXON, 1893: 188.

Munidopsis quadrata. – FAXON, 1895; pl. 23, Figs. 1, 1a-c. – BENEDICT, 1902: 325. – HART, 1982: 38. – WICKSTEN, 1989: 315. – HENDRICKX & HARVEY, 1999: 376.

Material examined. – St. 26-IV, 26/VIII/2000, 1 male (TL 31.0 mm), 1225-1240 m, BS.

Previously known distribution. – From Queen Charlotte Islands, Canada to Tres Marias Islands, Gulf of California, Mexico (WICKSTEN, 1989; HENDRICKX & HARVEY, 1999).

Remarks. – The rediscovery of *M. quadrata* in the southern Gulf of California confirms the presence of this temperate species in deep water of tropical Mexico. It is the first record of this species here since it was described by FAXON (1893) from 4 specimens captured at 1220-1225 m off Tres Marias Islands, Mexico. The male specimen reported here is slightly larger than the type material.

Other collected species

In addition to the 16 species treated above in the systematic section, the material from the TALUD IV cruise included nine species (see Table 3) that are briefly treated here. Two species of the strictly pelagic Sergestidae were captured. According to HENDRICKX & ESTRADA-NAVARRETE (1996), *Sergestes halia* Faxon, 1893, is a common species in the southern Gulf of California and extend throughout the eastern tropical Pacific. *Sergia phorca* (Faxon, 1893) shows a similar distribution pattern along the Pacific coast of America, but extends much further southwards, to southern Peru. *Gennadas sordidus* Kemp, 1910, is the only species of *Gennadas* known to the Gulf of California where it occurs abundantly except in the upper Gulf (HENDRICKX & ESTRADA-NAVARRETE, 1996). Adaptation of this species to low oxygen content in the water column consists of a strong increase of branchial surface (HANAMURA, 1983). The rest of the species (*Heterocarpus affinis* Faxon, 1893; *Acanthephyra brevicarinata* Hanamura, 1984; *Glyphocrangon spinulosa* Faxon, 1893; *Munidopsis diomedeae* (Faxon, 1893); *M. hystrix* Faxon, 1893; *Nephropsis occidentalis* Faxon, 1893) were also captured during the TALUD III cruise in the same area (Table 4).

TABLE 3

Species of decapod crustaceans collected during the TALUD IV cruise in the SE Gulf of California. Oxygen measured at bottom level. M = male; F = female; FF = ovigerous female; juv. = juvenile.

Species	Station	Depth	Oxygen content	Material examined (size)
Benthesicymidae				
Gennadas sordidus	13	1530-1520	1,46	1 F (CL 9.8 mm)
Gennadas sordidus	18	856	1,03	2 M (CL 9.0, 9.5 mm); 1 F (CL 9.8 mm)
Gennadas sordidus	19	1245-1240	0,73	1 juv. (CL 8.5 mm)
Benthesicymus tanneri	13	1530-1520	1,46	see text
Benthesicymus tanneri	19	1245-1240	0,73	see text
Benthesicymus tanneri	26	1225-1240	0,76	see text
Benthesicymus tanneri	33	1040	0,51	see text
Solenoceridae				
Hymenopenaeus doris	19	1245-1240	0,73	see text
Sergestidae				
Sergestes halia	4	1260	0,84	
Sergia phorca	26	1225-1240	0,76	1 F (CL 16.0 mm)
Pasiphaeidae				
Pasiphaea emarginata	25	870-835	0,29	see text
Pasiphaea magna	19	1245-1240	0,73	see text

Species	Station	Depth	Oxygen content	Material examined (size)
Oplophoridae				
Acanthephyra brevicarinata	19	1245-1240	0,73	1 M (CL 20.7 mm); 1 F (CL 28.1 mm)
Acanthephyra brevicarinata	20	1510	1,26	1 M (CL 27.8 mm)
Acanthephyra brevicarinata	26	1225-1240	0,76	2 FF (CL 22.0, 22.2 mm)
Acanthephyra brevicarinata	27	1550-1546	1,32	1 juv. (TL 45.0 mm)
Acanthephyra brevicarinata	33	1040	0,51	1 FF (CL 21.5 mm)
Acanthephyra brevicarinata	34	1240-1250	0,79	1 M (CL 11.2 mm)
Acanthephyra brevicarinata	35	2016-2020	1,68	1 F (CL 11.7 mm)
Acanthephyra brevirostris	26	1225-1240	0,76	see text
Hymenodora gracilis	35	2016-2020	1,68	see text
Nematocarcinidae				
Nematocarcinus cf. *ensifer*	13	1530-1520	1,46	see text
Nematocarcinus cf. *ensifer*	26	1225-1240	0,76	see text
Nematocarcinus aff. *ensifer*	26	1225-1240	0,76	see text
Nematocarcinus cf. *ensifer*	33	1040	0,51	see text
Hippolytidae				
Lebbeus scrippsi	19	1245-1240	0,73	see text
Lebbeus scrippsi	26	1225-1240	0,76	see text
Pandalidae				
Heterocarpus affinis	25	870-835	0,29	1 F (CL. 34.8 mm)
Heterocarpus affinis	33	1040	0,51	14 specimens (CL 23.5-38.5 mm)
Pandalus amplus	19	1245-1240	0,73	see text
Crangonidae				
Sclerocrangon atrox	13	1530-1520	1,46	see text
Sclerocrangon atrox	19	1245-1240	0,73	see text
Glyphocrangonidae				
Glyphocrangon sicaria	14	2160-2150	2,44	see text
Glyphocrangon sicaria	26	1225-1240	0,76	see text
Glyphocrangon spinulosa	19	1245-1240	0,73	2 F (CL 22.4, 22.9 mm)
Glyphocrangon spinulosa	26	1225-1240	0,76	3 F (CL 17.1, 17.9 mm); 1 FF (CL 26.6 mm)
Nephropidae				
Nephropsis occidentalis	19	1245-1240	0,73	2 M (CL 32.3, 33.2 mm); 1 F (CL 35.5 mm)
Nephropsis occidentalis	26	1225-1240	0,76	1 F (CL 33.3 mm)
Polychelidae				
Stereomastis pacificus	19	1245-1240	0,73	see text
Paguridae				
Parapagurus foraminosus	14	2160-2150	2,44	1 FF (CL 7.4 mm)
Galatheidae				
Munidopsis ciliata	19	1245-1240	0,73	see text
Munidopsis depressa	25	870-835	0,29	see text
Munidopsis depressa	26	1225-1240	0,76	see text
Munidopsis depressa	33	1040	0,51	see text
Munidopsis diomedeae	19	1245-1240	0,73	1 M (TL 65.0 mm)
Munidopsis diomedeae	20	1510	1,26	1 FF (TL 73.0 mm)
Munidopsis diomedeae	26	1225-1240	0,76	1 juv. (TL 19.9 mm)
Munidopsis hystrix	25	870-835	0,29	4 M (TL 48.4-51.5 mm)
Munidopsis hystrix	26	1225-1240	0,76	1 M (TL 59.0 mm)
Munidopsis palmatus	19	1245-1240	0,73	see text
Munidopsis palmatus	26	1225-1240	0,76	see text
Munidopsis quadrata	26	1225-1240	0,76	see text

TABLE 4

Species of decapod crustaceans collected during the TALUD III cruise in the SE Gulf of California. Species marked with an * have been reported in details elsewhere (see Hendrickx, 1996b).

Species	Station	Depth	Oxygen content (ml/l)
Benthesicymidae			
Benthesicymus tanneri	14A	1016-1020	0,40
Benthesicymus tanneri	14B	1188-1208	0,60
Benthesicymus tanneri	24A	1027-1060	-
Oplophoridae			
Acanthephyra brevicarinata	10A	956-980	0,25
Acanthephyra brevicarinata	14A	1016-1020	0,40
Acanthephyra brevicarinata	20A	880-1052	-
Acanthephyra brevicarinata	24A	1027-1060	-
Hippolytidae			
*Lebbeus scrippsi**	14B	1188-1208	0,60
Nematocarcinidae			
*Nematocarcinus cf. ensifer**	14A	1016-1020	0,40
*Nematocarcinus cf. ensifer**	14B	1188-1208	0,60
*Nematocarcinus cf. ensifer**	24	1224-1380	-
*Nematocarcinus cf. ensifer**	24A	1027-1060	-
Pandalidae			
Heterocarpus affinis	10A	956-980	0,25
Heterocarpus affinis	14A	1016-1020	0,40
Heterocarpus affinis	20A	880-1052	-
Heterocarpus affinis	24	1224-1380	-
Heterocarpus affinis	24A	1027-1060	-
Crangonidae			
*Paracrangon areolata**	14A	1016-1020	0,40
Glyphocrangonidae			
*Glyphocrangon spinulosa**	10A	956-980	0,25
*Glyphocrangon spinulosa**	24A	1027-1060	-
Nephropidae			
*Nephropsis occidentalis**	14B	1188-1208	0,60
Ctenochelidae			
*Callianopsis goniophthalma**	24A	1027-1060	-
Axiidae			
*Calocarides quinqueseriatus**	10A	956-980	0,25
Polychelidae			
Stereomastis nana	14B	1188-1208	0,60
Galatheidae			
*Munidopsis depressa**	10	820-826	-
*Munidopsis depressa**	10A	956-980	0,25
*Munidopsis depressa**	14A	1016-1020	0,40
*Munidopsis depressa**	14B	1188-1208	0,60
*Munidopsis depressa**	24A	1027-1060	-
Munidopsis diomedeae	19	1188-1208	-
*Munidopsis hystrix**	20A	880-1052	-
Atelecyclidae			
*Trachycarcinus corallinus**	14B	1188-1208	0,60

Oxygen content in the water column

Analysis of the dissolved oxygen concentration measured close to bottom (Fig. 4) indicated critical hypoxic conditions at depths between 300 and 800 m. Values higher than 1.0 ml O_2/l are found at ca. 1300 m and there is a clear tendency for oxygen content to increase significantly in deeper water. Values obtained during the TALUD IV cruise in August 2000 are similar, although slightly higher, to those registered at a depth range of ca. 400-1200 m during the TALUD III in August 1991 (Fig. 4). The vertical distribution of oxygen in the area (Fig. 5) indicates the presence

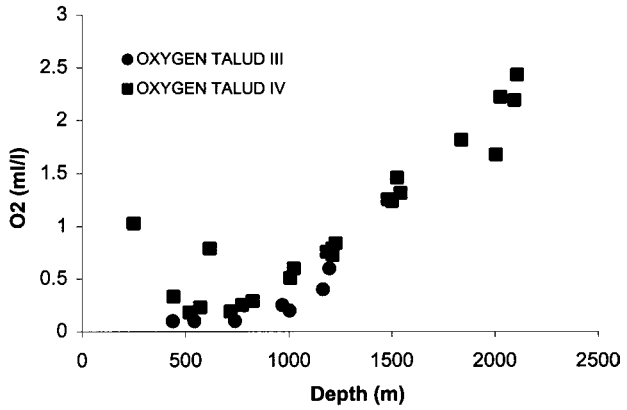

Fig. 4. – Dissolved oxygen concentration measured near bottom during TALUD III and IV cruises.

of three successive environments. The well-oxygenated epipelagic zone ranges from surface to about 80-125 m, with oxygen concentration as high or higher than 2 ml/l. Deeper into the water column, a wide hypoxic to almost anoxic mesopelagic zone is found, extending roughly from 150-200 m to 600-800 m (or to the bottom in stations shallower than 800 m). The deep water benthic-demersal environment is almost anoxic in localities shallower than 600-800 m but oxygen content is higher in deeper localities; oxygen reaches values of 0.5-1.0 ml/l in the depth range of 800-1300 m. In deeper water, there is a strong recovery of the oxygen content, which progressively reaches values above 2.0 ml/l in depths greater than 2000 m (Fig. 4).

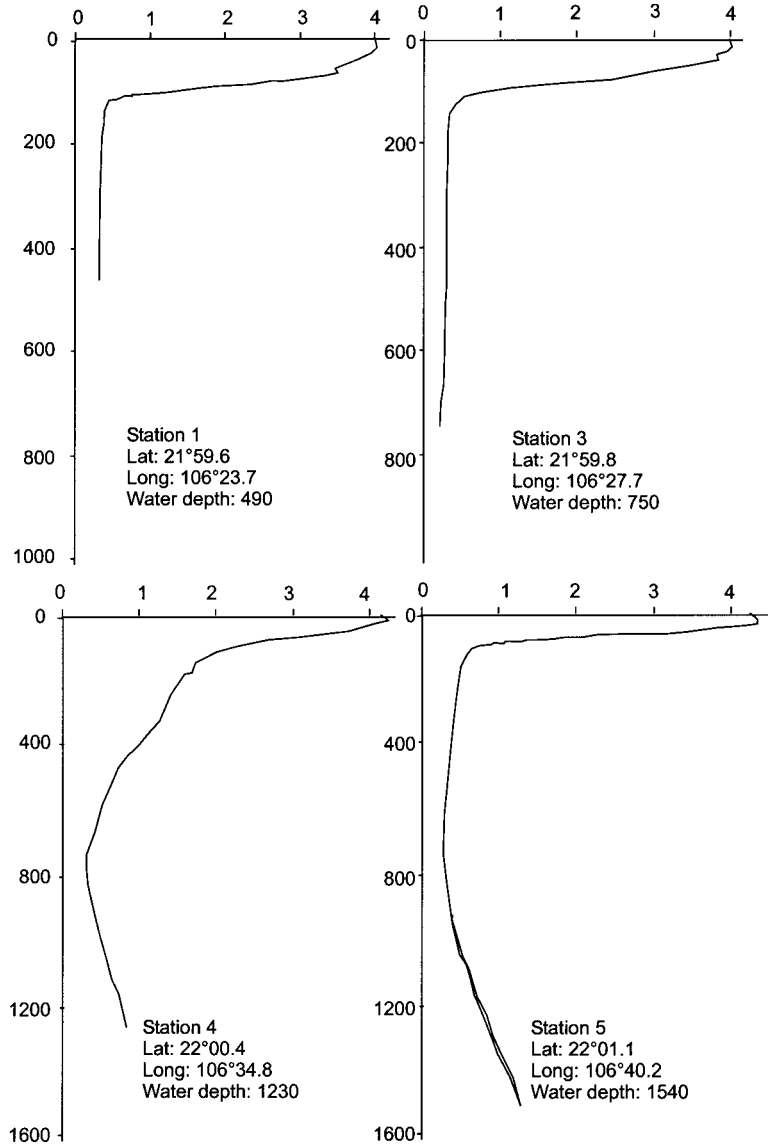

Fig. 5. – Vertical distribution of oxygen at selected stations; TALUD III (St. 9, 10A, 14, 14A, 14B and 15) and TALUD IV (St. 1, 3, 4 and 5) cruises.

Occurrence of deep-water species

A total of 31 species of decapod crustaceans living below 550 m was collected during both cruises (TALUD III, 15 species; TALUD IV, 26 species). Of these, 21 are typically benthic and eight are exclusively pelagic. Three species (*Benthesicymus tanneri*, *Acanthephyra carinata* and *Nematocarcinus* cf. *ensiger*) probably have a benthopelagic habitat as they have been collected with both benthic and pelagic gear; adults of these three species are heavy, and there might be a depth or habitat segregation among juveniles and adults. Six species of the deep water galatheid *Munidopsis* were collected. Considering the number of specimens collected, *M. depressa* appears as the dominant species (324 specimens at four stations) and no other species came near to this abundance. Four species were represented by 9-15 specimens during the entire 2000 survey (*Benthesicymus tanneri*, *Nematocarcinius* cf. *ensifer*, *Heterocarpus affinis* and *Acantephyra brevicarinata*) and the rest were even less abundant (Table 3 and text).

The number of species at each station varied considerably. During TALUD III cruise, the highest number of species obtained at one single station was eight (station depth: 1188-1208 m) and the lowest was one (Table 4). One station, at 820-826 m, yielded only one species of isopod. No strictly pelagic species were recorded. As

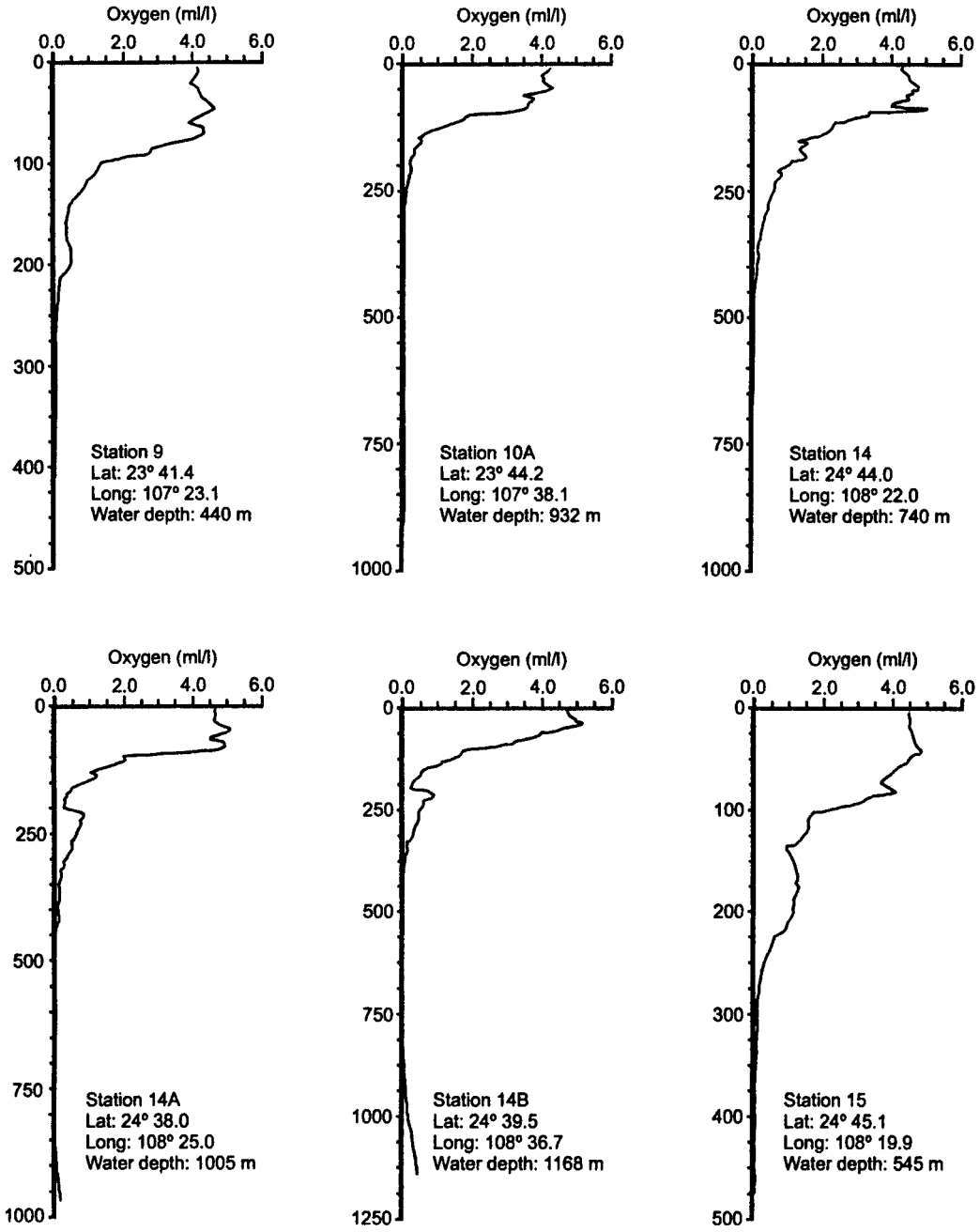

Fig. 6. – Interval of epibenthic oxygen content corresponding to species collected during the TALUD III and IV cruises.

noted previously, the second cruise (TALUD IV) yielded a considerably higher number of species. The highest number of species was obtained at station 19 (15 benthic and one pelagic species), followed by station 26 (12 benthic and one pelagic species) (Table 3). Coincidentally, both samples were obtained in the 1225-1245 m depth range and oxygen content was similar (0.73 and 0.76 ml 0_2/l). The highest number of species during TALUD III cruise was obtained in similar conditions: at 1188-1208 m, with oxygen content of 0.60 ml/l. Comparatively, the other stations feature a low species occurrence (Tables 3, 4). Oxygen content at bottom level does not seem to be a critical factor; indeed, the highest oxygen content during the TALUD IV cruise was measured at station 14 (2.44 ml O_2/l) but only two benthic species were caught. The other two stations where oxygen content was above 1.0 ml/l are stations 13 (1.46 ml/l) and 35 (1.68 ml/l only two benthic species were collected) in both stations. Two stations had four species and both feature a low oxygen content (0.29 ml/l at station 25 and 0.61 ml/l at station 33).

Results obtained during this survey allow us to determine the dissolved oxygen interval at which 21 species occur (Fig. 6), although for some species only one oxygen value is available. These intervals indicate that all these 21 species are occasionally found in hypoxic conditions, below 1.0 ml O_2/l.

Species occurrence with depth

Combined depth occurrence of species for both cruises indicates that all species, except the Pagurid, were collected at least once within the depth range of 835-1240 m (Fig. 7). Only five species (*A. brevicarinata*, *N.* cf. *ensifer*, *H. affinis*, *G. sicaria* and *M. diomedeae*) range to deeper water. The widest bathymetric range corresponded to *A. brevicarinata*. *Pasiphaea emarginata*, together with *P. magna*, and these were recorded in the pelagic realm. Typically benthic species *M. depressa*, *M. hystrix*, *G. spinulosa* and *H. affinis* feature a relatively wide bathymetric distribution and were rather common in samples (see Tables 3, 4 and text); they dominate the 800-1300 m decapod crustacean community.

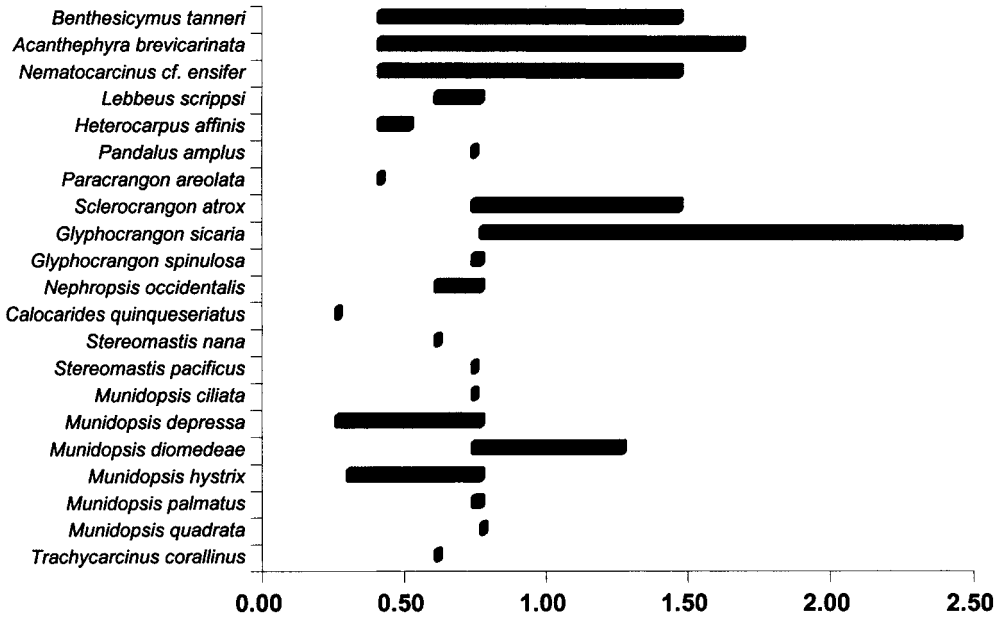

Fig. 7. – Bathymetric range observed for species collected during the TALUD III and IV cruises. (P) probably pelagic species.

DISCUSSION

The total number of species collected during both surveys is 31. Of these, 15 were described by W. FAXON on the basis of material collected by the *Albatross* in 1891. The new results indicate a higher species occurrence in the 1000-1380 m depth range, where hypoxic conditions still prevail, although measurements made near bottom level suggest a recovery in oxygen concentration at ca. 800 m. In the sampling area, oxygen content at bottom level does not seem to be a critical factor controlling species number, as expected. Fewer species were caught at stations with oxygen content in the range of 1.46-2.44 ml/l than at stations with much lower oxygen content. It might be concluded that, although critically low oxygen content (i.e., < 0.5 ml/l) may represent an impediment for the establishment of a rich benthic fauna, a higher oxygen content does not necessarily favour the presence of a richer fauna. Other factors such as availability of food, nature of substrate or strong submarine currents could affect species occurrence.

With epibenthic values close to 0.0 ml O_2/l (see HENDRICKX, 1995b), the oxygen minimum zone that extends off the coast of the southeastern Gulf of California represented an insuperable barrier for outer-shelf species. Even species known to tolerate low oxygen content (e.g., *Squilla biformis* Bigelow, 1891, *Solenocera mutator* Burkenroad, 1938, *Pleuroncodes planipes* Stimpson, 1860) are not found in deeper water in the area, despite the fact that there are deep water records of these species in other areas (*S. biformis*, to 518 m; *S. mutator*, to 360-380 m; *P. planipes*, to 366 m and exceptionally to 730 m) (HENDRICKX, 1995c, 1995d, 1995e). Not a single species belonging to the southeastern Gulf of California decapod crustaceans shelf community (107 species according to HENDRICKX, 1996a) was collected.

The bathymetric fringe extending roughly from 1000 to 1380 m presents a particular interest for its species richness. Food supply and trophic relationships among these species and with large, highly mobile predators (e.g., fishes and squids) that were not sampled during this survey, probably due to the type of sampling gear that was used, are major issues to be addressed. Other major issues include the accessibility to species with fishing potential (e.g., *Heterocarpus affinis*, *Pandalus ampla*, *Benthesicymus tanneri*) and the evaluation of standing stock, for which larger and faster sampling gear will have to be used.

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Feeding ecology of Konik horses and donkeys in Belgian coastal dunes and its implications for nature management

Eric Cosyns[1], Tine Degezelle[1,2], Else Demeulenaere[1,2] and Maurice Hoffmann[1,2]

[1] **University of Ghent, Dept. Biology, Research Group of Terrestrial Plant and Vegetation Ecology, K.L. Ledeganckstraat, 35, B-9000 Ghent, Belgium**
[2] **Institute of Nature Conservation, Research Group of Landscape Ecology and Nature Management, Kliniekstraat 25, B-1070 Brussels, Belgium**

ABSTRACT. Foraging behaviour and diet selection of Koniks and donkeys were studied in order to estimate their possible impact on vegetation development and hence their appropriateness as nature management tools.

Koniks show a larger intake rate and quantity than do donkeys. Koniks do not show significant seasonal differences in biomass intake, whereas donkeys consume significantly more in winter.

Both animal species feed mainly on graminoids. The Konik diet is composed of 86% of graminoids with an additional 12% of herbs. The donkey diet consists of 69 % of graminoids, which are mainly supplemented with browsing (18 %), e.g. twigs and leaves of *Ligustrum vulgare* and *Rubus caesius.*

Calamagrostis epigejos, Rosa pimpinellifolia (fruits), *Carex arenaria* and *Arrhenatherum elatius* are the most important plant species eaten by donkeys (based on number of bites and biomass). Koniks eat *Calamagrostis epigejos* significantly more, qualitatively (number of bites) as well as quantitatively (biomass intake), than any other plant species, but *Cirsium arvense, Calamagrostis canescens, Juncus subnodulosus, Holcus lanatus* and *Claytonia perfoliata* are also frequently consumed.

Koniks as well as donkeys do eat plant species that nature managers would like to see decline in dominance, e.g. *Calamagrostis epigejos*, but browsing on scrub species is insufficient to decrease the area occupied by shrubs.

KEY WORDS: foraging behaviour, diet selection, feeding preference, management, dunes, horse, donkey.

INTRODUCTION

During the 19th and the beginning of the 20th century grazing by domesticated livestock was a common practice in the coastal dunes (De Smet, 1961). Sheep, cattle, donkeys and horses grazed natural vegetation. For example in 1828 the dune area of the western Flemish coast (approx. 2500 ha) was grazed by 450 sheep, 240 cows, 112 donkeys and 51 horses. Wherever they appeared, scrub species were cut down and used as firewood. As a result a semi-natural landscape developed that was largely composed of a mosaic of white dunes, marram dunes, grey dunes, moist dune slack vegetation and dry dune grassland (Massart, 1908).

Corresponding author : E. Cosyns, e-mail : eric.cosyns@rug.ac.be

Since these agricultural practices were abandoned gradually during the middle of the 20th century shrub development increased. This led to a present-day scrub cover of about two thirds of the remaining open (not built-up) dune area. Among others the area of species-rich dune grassland decreased significantly. These trends, together with the increasing dominance of some competitive grasses e.g. *Calamagrostis epigejos, Arrhenaterum elatius, Elymus repens, Holcus lanatus*, are believed to threaten the relatively large number of dune specific species (van Dijk, 1992; ten Harkel & van der Meulen 1995). In addition, part of the landscape changed from a fine-scale mosaic of different habitats to a more or less monotonous shrub vegetation, which is relatively poor in habitat and in coastal dune specific plant and spider species (Provoost & Hoffmann, 1996[1]; Bonte et al., 2001[2]).

Since the legal protection of all Belgian coastal dune areas (Vlaamse Regering, 1993[3]), interest is growing in the possibility of using appropriate nature management to conserve at least the remaining biodiversity.

Because large herbivores formerly played an important role in the preservation of semi-natural dune communities (Westhoff, 1985; Hewett, 1985; Drost & Muis, 1988; van Dijk, 1992; van Deursen et al., 1993; Kooijman & van der Meulen, 1996), the Department of Nature of the Flemish Community decided to introduce cattle, horses, donkeys and sheep into some of their nature reserves.

To reach this goal, equids are considered to be interesting management "tools". Current knowledge about the feeding preferences of equids suggests that they should be very useful to control graminoids (Gudmundsson & Dyrmundsson, 1994). They should also affect some tree species (Van Wieren, 1987; Duncan, 1992). Their impact on herbs and shrubs on the other hand is considered to be lower than that of cattle (Duncan, 1992).

While some knowledge is available on the feeding behaviour of domesticated horses under semi-natural conditions in European ecosystems (Duncan, 1983; Duncan, 1992; Putman et al. 1987; Gordon, 1989), much less is currently known about an almost forgotten equid species, the domesticated donkey (*Equus asinus*) (Van Assche, 1993; Hoffmann et al., 2001).

To be able to predict the possible long-term effect of the feeding ecology of these large herbivores, we started a large-scale investigation into their diet preferences and their habitat use and location selection in some coastal dune areas. Here we describe some aspects of the foraging behaviour and the botanical characteristics of the diet of the domesticated donkey and the Konik horse (*Equus caballus*), a horse that is closely related to the Tarpan (*E. ferus silvaticus*).

MATERIAL AND METHODS

Sites, animals and management

In April 1997 a small herd of six donkeys (1 stallion, 5 mares) of Romanian origin was released for year round grazing in the nature reserve Houtsaegerdunes (80 ha).

In 1998 four Konik horses (2 stallions, 2 mares), and two Scottish Highland cattle were released also for year round grazing in the northern fenced area (54 ha) of the nature reserve, the Westhoek.

By March 2000 the Konik herd had grown with one 1999-born foal and the donkey herd then numbered 12 individuals (2 stallions, 7 mares and 3 foals).

The animals received no supplementary feeding. Water was available during the whole period at different sites in the study area.

Shrubs of *Hippophae rhamnoides*, *Ligustrum vulgare* and to a lesser extent *Salix repens* occupy the largest part of both dune areas. Before the start of the grazing project in the Westhoek 12% of the original 79 % shrub cover was cut down and removed, resulting in an area of ruderal vegetation composed of a low, grass-dominated layer and patches of tall herbs (*Eupatorium cannabinum*, *Lythrum salicaria* and *Cirsium arvense)*. Old, deteriorating *Hippophae*-scrubs are generally replaced by *Calamagrostis epigejos* or *C. canescens*. Dune grasslands, moss-rich grey dunes, open sand dune and young dune slacks together occupy another substantial part of both dune areas (Table 1). A typical phenomenon of the Houtsaegerdunes is the non-indigenous plant species, introduced in the past as hedge plants along small fields or escaped from neighbouring gardens (e.g. *Syringa vulgaris, Fallopia aubertii*).

Methods

Each month we observed herbivore activities during 48 hours, distributed more or less evenly over 6-hourly morning (6-12 h), afternoon (12-18 h) and evening (18-24 h) sessions. Before starting a session, 1 animal was randomly chosen to be followed for the next 6 hours. Observations were conducted within a 3-m range; animals were not visibly affected by the observations (after a fortnight of habituation to an observer).

Herbivore activities e.g. grazing (food intake), defecating, moving, standing inactive, lying and social interactions were recorded simultaneously by one observer in both areas.

During those observations we used continuous time registration with sessions subdivided in periods of 15 minutes, which is the smallest unit chosen for counting bites and calculating mean bite rates and bite frequencies. All plant species and plant parts seen bitten were recorded. Plant state (dead or alive) was also noted. Mixed bites were registered as different bites of one plant species but were counted only one time for bite rate calculation.

Finally we recorded in which vegetation community and vegetation height class (<10cm, 10-50cm and >50cm) activities occurred.

To estimate mean bite mass of the more frequently consumed plant species, bite simulations were conducted after every observation session. Plants or plant parts were hand-plucked using thumb and a backward bent forefinger, simulating the animals' grazing as closely as possible at the same place where the species was frequently seen bitten (Hobbs et al., 1983; Wallis de Vries, 1994). These samples, consisting of 10 times 30 bites of each plant item, were stored in paper bags, oven dried at 60 °C for 48 hours and weighed to get an estimate of bite mass. Together with the bite rate data, these bite size estimates were used to estimate intake (-rate) at the plant species level.

To investigate diet composition, diet preferences and temporal patterns in feeding ecology, we mainly used

TABLE 1

Main vegetation units of the 'Houtsaeger dunes' and the 'Westhoek noord' based on a vegetation analysis in 1998 respectively 1999 (VAN BRAECKEL unpubl. respectively DEVOLDERE & DEGEZELLE unpubl.).

Vegetation unit + code	Description	Area (ha) Houts.	Area (%) Houts.	Area (ha) West. N.	Area (%) West. N.
White dunes (A)	Open vegetation with *Ammophila arenaria*, *Carex arenaria*, *Festuca juncifolia*	2.69	3.6	2.2	4.11
Grey dunes (T)	Moss and Lichen rich dunes With scattered *C. arenaria* and therofytes.	4.67	5.87	2.8	5.24
Rough vegetations (U/C)	Grass layer (*Holcus lanatus*, *Poa trivialis*, *Claytonia perfoliata*) with scattered patches of tall herbs (e.g. *Eupatorium cannabinum, Cirsium arvense, Lythrum salicaria*)	none	5.55	10.36	
Ruderal vegetation (C5/U+R)	*Arrhenaterum elatius* dominated, with other grasses and *Urtica dioica, Rubus caesius and fruticosus*	4.78	6.01	none	
Dune grasslands (G)	Short grasslands with high plant diversity (e.g. dicotyledons)	0.93	1.17	2.19	4.09
Rose vegetation (I)	Dune grasslands dominated by *Rosa pimpinellifolia*	2.99	3.76	0.55	1.03
Dune-slack pioneer (J1/(S))	Short pioneer vegetation with *Carex* spp., *Juncus* spp. and young *Salix repens* and *Hippophae rhamnoides*	0.29	0.36	1.94	3.64
Rough dune-slack (J9/C1/C3)	Tall vegetation dominated by *Calamagrostis epigejos, C. canescens* and *Lythrum salicaria*	none		1.4	2.61
Reed	*Phragmites australis* dominated	0.23	0.3	none	
Deteriorating scrub (H/C1)	Dead scrub of *Hippophae rhamnoides*, grass layer dominated by *C. epigejos*	3.13	3.94	5.86	10.95
Scrub (L/H/S/P)	Scrub dominated either by *Ligustrum vulgare*, *H. rhamnoides, Salix repens* or mixed with other shrubs + sometimes herb layer with *Claytonia perfoliata*.	54.38	68.4	30.08	56.21
Wood (B)	*Populus* spp. or *Alnus glutinosa* dominated wood patches	4.64	5.8	0.16	0.31
paths	Pioneer vegetation of dry or wet situations	0.8	1	0.78	1.45
Total		**79.53**	**100**	**53.51**	**100**

ANOVA for testing significance of differences between means (F-test). Means were usually based on data at the 15-minute level. In case of inconsistency with the assumptions of ANOVA even after data transformation we used Kruskal-Wallis One way analysis (SOKAL & ROHLF, 1995; SIEGEL & CASTELLAN, 1988). For a test of normality and of homogeneity of variances we used respectively the Kolmogorov-Smirnov and Levenes test.

Multiple comparisons among means were carried out using an a posteriori HSD (equal variances assumed) or Games-Howel test (unequal variances) in SPSS 7.5 for Windows (NORUSIS, 1997).

To compare plant species preference we used the diet-availability ratio (COLEBROOK et al., 1987), discussed by STUTH (1991):

$$D/A = \{(\%\ \text{Diet} - \%\ \text{Availability})/(\%\ \text{Diet} + \%\ \text{Availability})\} * 10$$

STUTH (1991) used the following expressions for three different classes: preferred species: D:A > 0.35; desirable species: -0.35 < D:A < 0.35; undesirable, avoided or forced species: D/A < -0.35.

As a measure of diet we used the number of bites in summer, as a measure of availability we used the above ground biomass of every species in summer in the vegetation patches visited by the animals during the observation sessions (COSYNS & DEVOLDERE, unpubl.).

RESULTS

Bite rate and bite frequency

Bite rate (bites/min. grazing) of Koniks is significantly higher than that of donkeys. This is the case over all seasons with the greatest difference in summer and the smallest in winter (Table 2). Bite rate of both animals varies with seasons. Koniks graze substantially faster in summer than in autumn and winter ($p < .001$). Bite rate of donkeys shows the opposite trend; winter bite rate differs significantly from bite rate in summer and autumn ($p < .001$).

Bite frequency (Bites/min. observation time) of Koniks does not show any significant difference between seasons. On the contrary, donkeys reach a substantially higher bite frequency in winter than they do in the other two seasons ($p < .001$) (Table 3).

Koniks spend significantly more of their time grazing (73%) than do donkeys (52%) ($p < .001$). Neither Koniks nor donkeys show a significant seasonal variation in grazing pattern, although donkeys tend to increase grazing time from summer to winter (Fig. 1).

TABLE 2

Variation in mean bite rate (bites/ min. foraging time) of konik and donkey per season. All results are significantly different between both herbivores within seasons (columns) ($p<.001$).

(Bites/min. grazing)	Seasons		
Animal	**Summer**	**Autumn**	**Winter**
Konik	33.74	24.10	26.28
Donkey	10.41	12.59	18.13

TABLE 3

Bite frequency (bites/min. observation time) of konik and donkey per season. Bite frequency is used as a preliminary measure for their intake. Therefore mean bites/min. observation time is compensated for differences in mean bite rate between seasons. Significantly different results ($p < .001$) between periods are indicated (***).

(Bites/min. observ.)	Seasons		
Animal	**Summer**	**Autumn**	**Winter**
Konik	20.12	18.66	20.05
Donkey	6.30	6.80	8.60 (***)

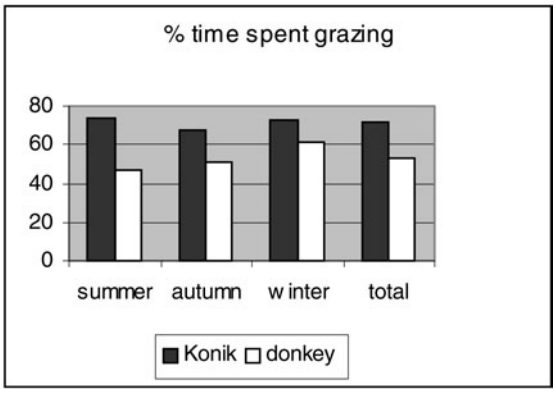

Fig. 1. – Mean grazing time of konik and donkey as % of total observation time/season. Koniks spend significantly more of their time grazing than donkeys ($p<.001$). Koniks do not show significant seasonal differences. Donkeys tend to increase foraging time from summer to winter (but $p=0.052$, F-test).

Forage class

We observed significant differences ($p < .001$) among animal species, although they both feed mainly on grasses and grass-like species. The diet of the Konik horse is composed of 86% grasses, the remainder being mainly herbs e.g. *Cirsium arvense*, *Stellaria media* and seedlings of *Claytonia perfoliata* (12%). Woody species (mainly *Rubus caesius*) are only consumed in small amounts. Graminoids were eaten significantly more in summer than in autumn or winter ($P< .001$). Herbs (mainly *Claytonia* and *Stellaria*) were eaten substantially more in winter (Table 4).

TABLE 4

General diet composition (% of total number of bites) of konik and donkeys in two Belgian coastal dune nature reserves during summer-winter 1999-2000. Overall number of bites (compensated for differences in observations) is significantly different between both equid species ($p<.001$).

Forage class	Summer	Autumn	Winter	Total (***)
Konik				
Graminoids	93.6	87.1	80.6	86
Herbaceous plants	4.4	11.2	18	12
Woody plants (browse)	2	1.7	1.4	2
Donkey				
Graminoids	60.6	79.5	86	69
Herbaceous plants	10.4	7.4	6.6	13
Woody plants (browse)	29	13.1	7.4	18

The donkey's diet consists of 79 % graminoid species, the remainder provided mainly from browsing of woody species, e.g. twigs and leaves of *Ligustrum vulgare* and *Rubus caesius,* fruits of *Rosa pimpinellifolia* and *R. canina* (13%). Woody material was eaten more in summer and autumn than in winter ($p< .001$). Herbs were the smallest part in this diet (8%) and were mainly eaten in winter (*Claytonia perfoliata*).

Plant species

Koniks and donkeys consume a wide variety of plant species. During the whole observation period Koniks ate 89 plant species: 24 graminoid species, 54 herb species and 11 woody species.

During the same period donkeys ate 111 plant species: 18 graminoid species, 63 herb species, 27 woody, 1 fern,1 lichen and 1 moss species.

In both cases about one third of all plant species known in the respective study areas were bitten.

With mean bites/min. foraging time as the criterion, the Konik diet over the whole period was mainly composed of grasses. *Calamagrostis epigejos, Poa trivialis* and

Holcus lanatus were bitten substantially more than all other plant species. *Claytonia perfoliata*, *Stellaria media*, *Calamagrostis canaescens* and *Juncus subnodulosus* make up a second group of frequently bitten plant species. (Table 5).

When mean intake rate is the criterion, *Calamagrostis epigejos* is still the most important species (P<.001) but Koniks seems to eat also considerable amounts of *Cirsium arvense* (Table 5).

Donkeys frequently consume *Festuca juncifolia, Calamagrostis epigejos* and *Carex arenaria* and, to a significantly lesser extent, also *Arrhenaterum elatius*, *Claytonia perfoliata*, fruits of *Rosa pimpinellifolia* and *R. canina*, *Holcus lanatus*, *Elymus repens* and leaves and twigs of *Fraxinus excelsior* and *Ligustrum vulgaris* (Table 6).

When comparing the plant species preferences of both equids, using the D/A classification designed by STUTH

TABLE 5

Konik diet. The twelve most bitten plant species in the northern fenced part of the nature reserve the Westhoek. Mean bite rate is indicative for the most frequently bitten species. Over the whole period these are *Calamagrostis epigejos*, *Poa trivialis* and *Holcus lanatus* (HSD results are given underneath the diagonal). Zero-bite observations per species are left out of calculation of the mean assuming that during these zero-bite periods the plant species were not met with by the Konik. With mean intake rate (dry matter/min. grazing) as criterion *Calamagrostis epigejos* is by far the most important plant species consumed (HSD test right side). (***: p<0.001;**; p<0.01; p<0.05; ns: p >0.05). Empty cells indicate a lack of information.

Species	Mean B/min	DM g/min	1	2	3	4	5	6	7	8	9	10	11	12
1. Calamagrostis epigejos	8.43	2.04	1		***		***	***	***					***
2. Poa trivialis	7.81	NA	ns	1										
3. Holcus lanatus	7.4	1.39	ns	ns	1		ns	ns	ns					ns
4. Stellaria media	5.41	NA	***	***	ns	1								
5. Claytonia perfoliata	4.53	0.7	***	***	***	ns	1	ns	ns					ns
6. Juncus subnodulosus	4.21	0.7	***	***	***	ns	ns	1	ns					ns
7. Calamagrostis canescens	3.39	1.02	***	**	***	ns	ns	ns	1					ns
8. Carex arenaria	2.1	NA	***	***	***	*	ns	ns	ns	1				
9. Agrostis stolonifera	1.54	NA	***	***	***	***	*	ns	ns	ns	1			
10. Eupatorium cannabinum	1.28	NA	***	***	***	***	***	**	ns	ns	ns	1		
11. Rubus caesius	0.88	NA	***	***	***	***	***	***	ns	ns	ns	ns	1	
12. Cirsium arvense	0.87	1.35	***	***	***	***	***	***	ns	ns	ns	ns	ns	1

TABLE 6

Donkey diet. The twelve most bitten plant species in the nature reserve the Houtsaegerdunes. Zero-bite observations per species are left out of calculation of the mean assuming that during these zero-bite periods the plant species was not met with by the donkey. Bite rate is indicative for the most frequently bitten species. Over the whole period these are *Festuca juncifolia*, *Calamagrostis epigejos* and *Carex arenaria*. With mean intake rate (dry matter/min. grazing) as criterion Calamagrostis epigejos is by far the most important plant species consumed (HSD test right side). (HSD ***: p<0.001;**; p<0.01; p<0.05; ns: p >0.05).

Species	Mean B/min	DM g/min	1	2	3	4	5	6	7	8	9	10	11	12
1. *Festuca juncifolia*	9.56	0.7	1	***	ns	***	ns	ns	***			ns	ns	ns
2. *Calamagrostis epigejos*	6.84	6.36	ns	1	***	***	***	***	ns			ns	***	***
3. *Carex arenaria*	6.04	0.42	ns	***	1	***	ns	ns	***			ns	***	***
4. *Arrhenaterum elatius*	5.26	3.1	***	ns	ns	1	***	***	ns			ns	***	*
5. *Claytonia perfoliata*	4.37	0.48	ns	***	ns	ns	1	ns	***			ns	ns	*
6. *Rosa pimpinellifolia* (fruit)	3.70	1.11	***	***	ns	ns	ns	1	***			ns	ns	ns
7. *Rosa canina* (fruit)	2.63	4.83	***	***	ns	ns	ns	ns	1			ns	***	*
8. *Holcus lanatus*	2.61		***	***	***	ns	ns	ns	ns	1				
9. *Elymus repens*	2.48		***	***	***	ns	ns	ns	ns	ns	1			
10. *Fraxinus excelsior*	2.43	3.4	***	***	***	ns	ns	ns	ns	ns	ns	1	ns	ns
11. *Ligustrum vulgare*	2.43	1.05	***	***	***	ns	ns	ns	ns	ns	ns	ns	1	ns
12. *Ammophila arenaria*	1.98	1.68	***	***	***	***	ns	ns	ns	ns	ns	ns	ns	1

(1991), we found some similarities but also some striking differences. Koniks prefer *Holcus lanatus, Calamagrostis epigejos* and desire *Rubus caesius,* whereas *Eupatorium cannabinum, Cirsium arvense* are undesirable (Table 7). Donkeys prefer *Carex arenaria* and desire *Calamagrostis epigejos* and *Avenula pubescens.* Undesirable to donkeys were e.g. *Arrhenaterum elatius, Rubus caesius, Ammophila arenaria, Festuca rubra* and *Achillea millefolium*.

TABLE 7

Plant species preferences of konik and donkey expressed by the diet-availability ratio (COLEBROOK et al., 1987).
Only those Plant species of which the total available biomass exceeds 1% of the total biomass in the area are taken into account.

Preferred D/A > 0.35	
Konik	**Donkey**
Holcus lanatus *Calamagrostis epigejos*	*Carex arenaria*
-0.35 < Desirable D/A ≤ 0.35	
Rubus caesius	*Calamagrostis epigejos*
Undesirable D/A ≤ -0.35	
Eupatorium cannabinum *Rosa pimpinellifolia* *Cirsium arvense*	*Avenula pubescens* *Arrhenaterum elatius* *Ammophila arenaria* *Rubus caesius* *Achillea millefolium*

Plant parts and plant state

Green leaves are by far the most bitten plant parts by both herbivore species (Table 8). This is certainly true for all grass (-like) species, but not necessarily the case for herbaceous or woody plant species. For example donkeys prefer fruits of *Rosa* spp. and the inflorescences of *Hieracium umbellatum*, *Melandrium album* and *Eupatorium cannabinum* above their foliage.

Koniks were seen biting inflorescence, young leaves and shrivelled plants of *Cirsium arvense,* inflorescences of *Eupatorium cannabinum* and, to a much lesser extent, fruits of *Rosa pimpinellifolia* and *Rubus caesius.* During winter Koniks not infrequently dig up and consume roots and rhizomes of *Urtica dioica* and *Epilobium hirsutum*.

DISCUSSION

Temporal feeding behaviour

As hindgut fermenters equids have to spend a lot of their time foraging (DUNCAN, 1992, ILLIUS & GORDON, 1993). Free-ranging horses devote 50-70% of their time to eating and only 20-30% to resting. Towards the autumn the time spent grazing increases (GUDMUNDSSON & DYRMUNDSSON, 1994). However, the increase in foraging time is limited. Camargue horses -although nutritionally stressed at the end of the winter show only a slight increase of 6 % in feeding time, suggesting a certain threshold above which further increase in feeding time would not outweigh the costs of sleep deprivation or fatigue (DUNCAN, 1992).

Our results with Koniks are to some extent in agreement with these conclusions, although grazing time in summer and autumn appears to be only slightly greater (3%) than time spent grazing in winter. Perhaps Koniks are at the border of feeding capacity in the winter- not being able to enlarge consumption anymore. The rather poor condition of one of the lactating mares in winter can be interpreted as a first signal for nutritional stress and the inability to increase intake for maximum nutrient assimilation. We therefore hypothesise that the feeding strategy of Koniks is based on high intake when food items are of high quality and best available (late spring, summer and early autumn) and that they rely upon their body reserves during periods of inadequate food availability.

TABLE 8

Konik and donkey diet composition at the plant part level (% of total number of bites)

Forage class	leaf	stem	Flower	fruit	seedling	root	bark
	Konik						
Graminoids	73.07	0.01	0	0	0	0	0
Herbaceous plants	9.79	7.19	0.44	0.07	7.25	0.17	0
Woody plants	0.95	0.72	0.01	0.23	0.03	0	0.07
Total	***83.81***	***7.92***	***0.45***	***0.3***	***7.28***	***0.17***	***0.07***
	Donkey						
Graminoids	71.71	0.27	0.42	0.29	0.19	0.01	0
Herbaceous plants	5.91	5.46	0.40	0.26	0.01	0.03	0
Woody plants	6.49	4.78	0.06	3.41	0.01	0	0.29
Total	***84.11***	***10.51***	***0.88***	***3.96***	***0.29***	***0.04***	***0.29***

Donkeys increase their intake significantly in winter and hence are able to maintain good condition. Donkeys are capable of consuming fibre at a high rate because of an efficient tooth and jaw apparatus and an ability to swallow larger feed particles (MUELLER et al., 1998). Donkeys are also known to be capable of digesting low quality food. Compared to horses, they have lower energy requirements (IZRAELY et al., 1989a; IZRAELY et al., 1989b). We suggest that a combination of these factors makes it profitable for them to feed more in winter. So donkeys seem to behave in a slightly different way when faced with decreased quality but still adequate quantities of food.

Botanical aspects of the diet

Free ranging horses consume a wide variety of plant species and are seasonally dependent in their selection. The availability of plant species has a great influence on their selection (GUDMUNDSSON & DYRMUNDSSON, 1994). Horses prefer grasses and other graminoid species above herbaceous species that have a larger amount of less favourable secondary compounds (PUTMAN et al., 1987; GORDON, 1989; DUNCAN, 1992; GROOT BRUINDERINK et al., 1997). Diet selection by Koniks and donkeys is quite similar. They seem to select first those graminoid species that are common and widespread. When it is possible, they can be very selective, consuming leaves and twigs, flowerheads or fruits of different herbaceous or woody species, which perhaps offer them some indispensable nutrients. At such times donkeys seem to prefer woody as well as herbaceous species whereas Koniks seem to select almost only herbaceous species. Reasons for that remain unclear. Nevertheless many herbaceous species are almost not or never eaten presumably because of secondary compounds or structural defences.

So far both animals can be considered as interesting nature management 'tools':

First of all Koniks as well as donkeys eat dominant plant species that nature managers would like to see decline in dominance, e.g. *Calamagrostis epigejos, Arrhenaterum elatius* and *Cirsium arvense*. However, browsing on scrub plants is insufficient to cause a visible decrease in their presence. Only some trimming effect and ring barking are achieved by the donkeys, while the Koniks have no foraging impact on scrub plants whatsoever. This minor impact of both equids on woody species might, however, result from the relative abundance of the more preferred graminoid species. In other areas, where graminoid presence is limited, donkeys have had considerable impact on woody species (VAN ASSCHE, 1993; VELTER, pers. comm.).

Within the given circumstances of relatively low-productive dune ecosystems, both animal species seem to perform well. Generally they cope well with periods of scarcity of food resources. However, they use different feeding strategies, presumably based on physical and physiological differences.

Before deciding on herbivore species and densities to be used for specific management goals, the feeding ecology of other large herbivores and of the effects of increasing animal densities on animal diet selection and vegetation dynamics need further attention. Clearly further assessment of food quantity and quality is inherent within this kind of research.

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Feeding behaviour in the bumble bee *Bombus terrestris*

Paul Smeets and Marie José Duchateau

Utrecht University, Faculty of Biology, Social Ethology group
P.O.Box 80.086, NL-3508 TD Utrecht, The Netherlands

ABSTRACT. Bumble bees (*Bombus terrestris*) are social insects that live in one-year colonies. Larvae are fed progressively by the workers up until the moment they pupate and transform into adults. An elaborate hypothetical scheme concerning the dynamics of feeding behaviour is presented. The central hypothesis is that larvae emit a hunger-signal that can inform workers about their nutritional status and thereby influence the feeding behaviour of the workers. In addition it is hypothesized that the receptivity of the workers for the hunger signal and their motivation also influence their decision whether or not to go and feed larvae. This contrasts with the prevalent view that workers impose a feeding regime on larvae, who passively undergo their rearing. Worker (feeding) behaviour in a number of colonies was recorded and experiments were conducted in order to elucidate several aspects of the dynamics of feeding behaviour. In the experiments presented here the strength of the hunger signal was manipulated in various ways: by starving larvae, feeding artificial food to larvae in vivo, and varying the number of larvae. The results of the experiments indicate that indeed in *B. terrestris* larvae emit a short-range hunger-signal that can be perceived by workers and that can trigger worker behaviour such as "long pollen eating" (which usually precedes feeding) and feeding larvae. This strongly suggests that a feeding regime is not simply imposed on larvae by workers. However, the motivation of workers also plays a decisive role in feeding behaviour.

KEY WORDS: *Bombus terrestris*, bumble bee, feeding behaviour, pollen eating, larvae, hunger signal, caste determination.

INTRODUCTION

Bumble bees are social insects that live in one-year colonies. The queen lays the eggs and the workers perform all necessary duties, among others foraging and feeding larvae. In *Bombus terrestris* (Latreille) larvae are fed progressively with a mixture of pollen and nectar plus some glandular secretions (PEREBOOM, 2000). At the colony level the feeding rate seems to be well regulated (PENDREL & PLOWRIGHT, 1981). At the level of individual larvae, however, regulation of the feeding rate appears relatively poor; the time between successive feedings of a larva varies considerably (PENDREL & PLOWRIGHT, 1981; RIBEIRO, 1999).

RÖSELER & RÖSELER (1974) refer to several authors who reported that in *Bombus* species some larvae are fed more than others, e.g. because of their position in the brood clump, and that this determines their final size. The prevalent view is that the workers impose a feeding regime on the larvae who passively undergo their rearing (RÖSELER, 1970, 1991; see also PLOWRIGHT & JAY, 1977). Contrasting with this view is evidence from PEREBOOM (1997) that workers are able to perceive the nutritional status of larvae and adjust their behaviour accordingly: He showed that starved larvae are fed more often than non-starved larvae. In addition, PEREBOOM (1997) found that larvae ingest food on their own account, and are capable of refusing food. These findings suggest a more active role of the larvae.

Before being able to feed, a worker needs to drink nectar and eat pollen. DUCHATEAU (unpublished data) found that workers who fed larvae after pollen eating ate pollen significantly longer than workers that did not feed after pollen eating (workers were observed for 30 minutes after they had stopped eating pollen). She also found that workers who were going to feed larvae spent more time on the

Corresponding author: M.J. Duchateau,
e-mail: M.J.H.M.Duchateau@bio.uu.nl

broodnest and more time manipulating the larvae's wax envelopes than workers who were not going to feed.

The work of PEREBOOM (1997) and DUCHATEAU (unpublished data) strongly suggests that workers initiate pollen eating and feeding in response to information they perceive concerning the nutritional status of larvae. Both PEREBOOM (1997) and RIBEIRO (1997) suggested that larvae produce some kind of stimulus that elicits feeding behaviour. On the basis of their work we hypothesized that larvae emit a signal that can inform workers about their nutritional status ("hungryness"). The fact that there is considerable variation in the characteristics of the feeding behaviour of an individual worker (PENDREL & PLOWRIGHT, 1981; RIBEIRO, 1997, 1999; PEREBOOM, 1997) is an indication that receptivity for the hunger signal and the motivation to feed of the workers also play a role in feeding behaviour. In addition, many other factors may influence the dynamics of feeding behaviour. On the basis of literature and our hypotheses we made a schematic representation of the dynamics of feeding behaviour (Fig. 1). Several experiments were conducted to test the validity of this scheme. In this paper, experiments investigating the presence of the hunger signal are presented. This was done by starving larvae, manually feeding larvae in vivo and varying the number of starved larvae.

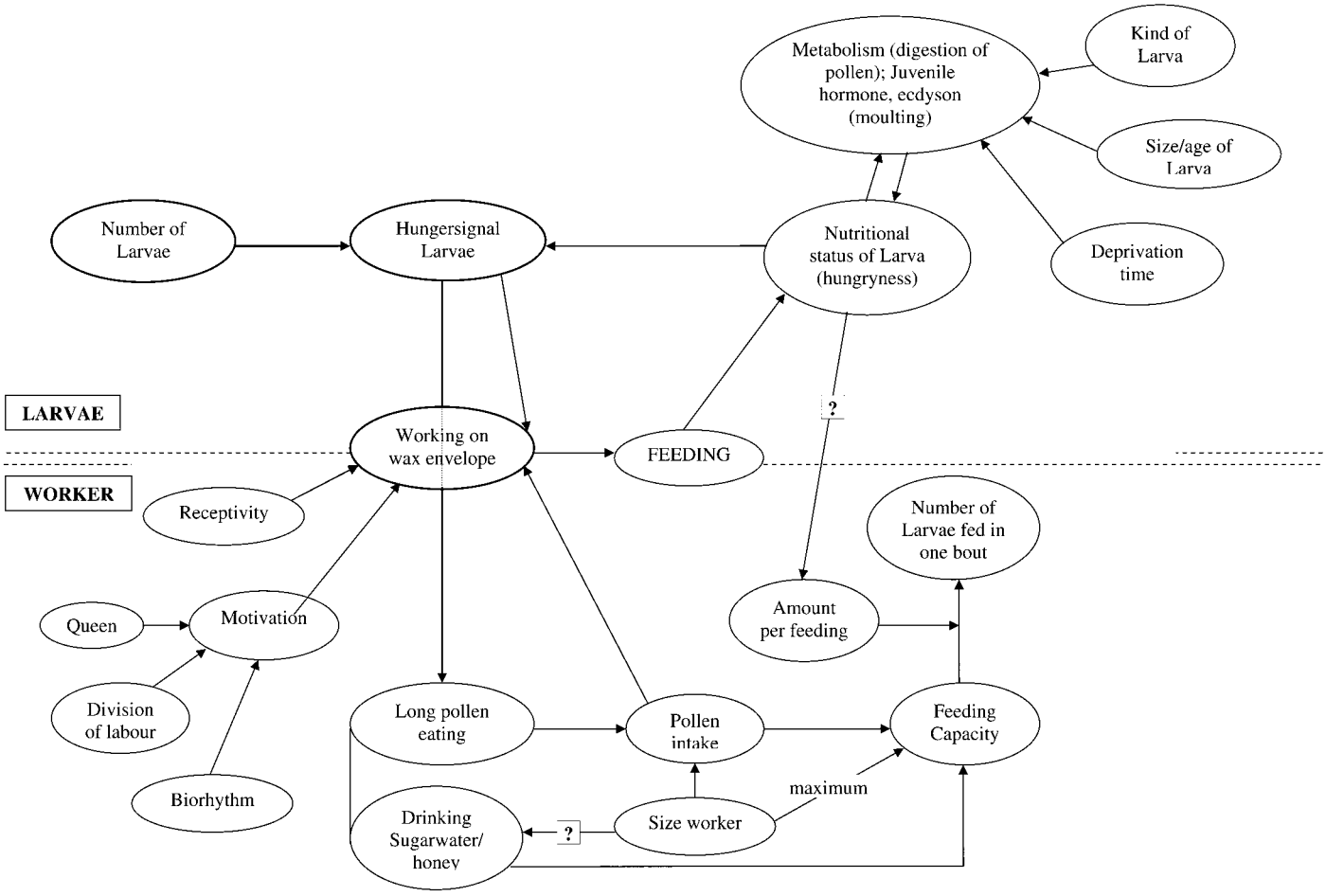

Fig. 1. – Hypothetical schematic representation of the dynamics of feeding behaviour.

MATERIAL AND METHODS

All observations and experiments were conducted under standard laboratory conditions in a climate-controlled room (28°C, 60% rH), illuminated by red light. Colonies of *Bombus terrestris* were reared and kept in the laboratory under the same conditions and provided with ad libitum pollen and sugar water (DUCHATEAU & VELTHUIS, 1988). Data were transformed and analyzed using Microsoft Excel 97 and SPSS 9.0.0 for Windows.

Observations of feeding behaviour in a colony

Young colonies were placed in an observation room (28°C, 60% RH) and connected to a small flight cage (40∗50∗66 cm) with a plastic tube (inner ∅ 15mm). The flight cage was covered by a non-transparent cloth and illuminated by a UV-lamp (TL/05 40 Watt) from 9.30 hours till 21.30 hours. A container with sugar water (1:1) was provided in the flight cage, ad libitum pollen in the nest box. Observations started as soon as the bees had

learned to forage for the sugar water. Non-foraging workers were selected and marked by glueing a small numbered tag on their thorax. Marked workers were followed for at least 15 minutes until one of them started eating pollen. From that moment on all behaviour of this worker was continuously recorded for at least 90 minutes according to an elaborate ethogram using The Observer version 3.0 (Noldus Information Technology 1994). For the sake of simplicity, only one behavioural state was active at a time. If, at the end of 90 minutes of observation, the worker had just eaten pollen or was in the middle of a feeding bout the observation was continued up to 120 minutes. In this way 13 different workers from six colonies were observed. One worker was observed ten times, the others one, two or three times depending on whether or not they were the first of the marked workers in a colony to start eating pollen. The total number of observation sessions was 29.

From the observational data, the duration of each behavioural element was calculated. Later, several intervals related to feeding behaviour, such as the time between the end of a Pollen Eating (PE) session and the first feeding, and the time between the last feeding in a bout and the next PE-session, were calculated. If the time between two instances of pollen eating (PE) was smaller than 30 s, the durations of the two PE-sessions were lumped and counted as one PE-session. If the time between two instances of pollen eating exceeded 20 minutes (1200 s) and no larvae were fed, the two PE-sessions were considered separate sessions. If the time between two instances of PE was between 30 s and 20 minutes (1200 s) and no larvae were fed, the durations of PE were lumped but counted as two sessions (of one "pollen-eating bout").

Feeding behaviour has the following characteristics: a worker (or queen) manipulates the wax envelope surrounding (a clump of) larvae and makes a small opening with her mandibles, if necessary. Then she inserts her mandibles, antennae, and part of her head, and after 0.5 up to about 10 seconds of "positioning" she sits motionless for a short time (0.5-5 seconds) and subsequently regurgitates a droplet of food from her honey stomach onto the ventral side of the larva by contracting and/or elevating her abdomen. After that, she either closes the orifice or manipulates it for some time (see also KATAYAMA, 1973, 1975; RIBEIRO, 1999). The feeding behaviour as described above is usually repeated several times in a short period of time, comprising a feeding bout (KATAYAMA, 1973, 1975 for *B. ignitus* and *B. hypocrita*; PENDREL & PLOWRIGHT, 1981).

Choice experiment starved/non-starved larvae

A standard observation box (20x30x7 cm) was divided into three compartments by two pieces of metal grid. In one of the grids there was a small flexible piece allowing the experimenter to open and close a small door. In the middle compartment sugar water and pollen were available.

Larvae of roughly similar size (aged 4-7 days) were taken from several colonies and placed in groups of ten to 12 in flat cups constructed from bee wax. The cups were covered with a thin layer of the larvae´s own wax envelope and, if there was not enough of that, with involucrum, in order to mimic a natural group of larvae. The cups were placed two by two in boxes with five workers that were seen to manipulate wax in a colony, allowing the workers to "remodel" the wax covering of the cups. In the first trial, half of the cups were put separately in a box after several hours, starving them overnight (14-17 hours). In the second trial, sugar water and pollen were provided initially but the pollen was taken away from half of the boxes for the night to prevent the workers from feeding larvae. The next day a group of starved and a group of non-starved larvae were placed in one of the outer compartments of the observation box, alternately left and right of the small door, about 2 cm from the metal grid.

In the first trial, one worker that was observed to feed larvae was taken from a colony and put in the middle compartment. After 15 minutes of habituation the small door was opened allowing the worker to access the compartment containing the two groups of larvae. For the following 10 minutes it was recorded on which group of larvae the worker was present and for how long. Also the occurrence of feeding and the first choice (the group of larvae she walked on first) of the worker was recorded. Then the observation box was cleaned with wet tissue to remove possible scent marks. After 3-4 hours the group of non-starved larvae was replaced by a new one. This procedure was repeated 25 times in 3 days.

In the second trial a group of five workers that were observed pollen eating or sitting on the main pollen store (located in a petri dish 5 cm in diameter) were taken from a colony and placed in the main compartment of the observation box. After the small door was opened, the occurrence of feeding and the first choice of workers were recorded. This procedure was repeated 30 times in 3 days.

Manually feeding a group of larvae in vivo

Twelve queen larvae in two different colonies were used. Six randomly chosen queen larvae were manually fed artificial food during 2 hours. This was repeated three times. During the experiment feedings by workers to these larvae and six control larvae were recorded. The artificial food consisted of a mixture of 10 g glucose and 40 g fructose filled up to 100 ml with tap water plus 1/3 volume of pollen (PEREBOOM, 1997). On two days the manual feeding regime was 19µL every 20 minutes, on one day 9µL every 10 minutes for 1 hour and then 3.5µL every 5 minutes for the second hour.

Varying the number of larvae

Larvae aged 5-7 days were taken from colonies and put into flat cups constructed of bee wax in groups of approximately five or approximately 15 larvae using the same procedure as in the choice experiment described before. When larvae had recuperated sufficiently, the cups were put apart from the workers and starved overnight (16-20 hours). Then, a feeding worker was obtained from a colony. Her abdomen was pressed gently so as to remove the food store in her honey stomach. Subsequently she was placed in a standard observation box with a cup of starved larvae and provided with sugar water. After 1.5-2 hours of habituation, pollen was provided and the pollen dish and the larvae were videotaped for 6-9 hours using a Euromex tablecamera. Afterwards the tapes were analyzed recording all instances of Pollen Eating and Feeding larvae. In this way 11 sessions were done with groups of about five larvae of which ten sessions were used for further data analysis (one session yielded no data). Thirteen sessions were done with groups of about 15 larvae of which 11 sessions were used for further data analysis.

RESULTS

Feeding behaviour in a colony

In order to get a detailed impression of feeding behaviour, individual workers in a colony were observed continuously for 90 minutes or longer. Here, only the duration of Pollen Eating (PE) and the number and timing of feedings will be presented.

PE not followed by Feeding (FE) consisted of one eating session in seven out of nine cases (78%). The mean frequency of PE not followed by feeding was 0.24±0.51 times per hour. The mean duration of PE not followed by feeding was 72±79s.

PE followed by FE was much more frequent, on average 1.85±1.53 times per hour and consisted of one eating session in 27 out of 44 cases (61%). In the other cases (39%) PE followed by FE consisted of more than one eating session. Thirteen out of these 17 "eating bouts" (76%) consisted of two sessions (30% of the total, 30s<interval time<1200s). The mean duration of PE followed by FE was 287±187s. This was significantly longer than the mean duration of PE not followed by feeding (Mann Whitney U test $p<0.01$, n=9 and n=48 respectively). This confirms the finding of DUCHATEAU (unpublished data) that on average workers eat pollen significantly longer before feeding larvae.

Choice experiment starved/non-starved larvae

To investigate whether or not workers are able to discriminate between starved and non-starved larvae from a distance of 2 cm, two choice experiments were conducted. One using one worker and one using a group of five workers. Table 1 shows the first choice of workers in both experiments. Clearly, the first choice of workers is not biased. Also, the mean time workers spent on the broods in the one worker experiment did not differ between the two broods (starved: mean 167±199s, non-starved: mean 241±214s, paired samples t-test, n=25, p=0.334). A similar result was obtained for the five worker experiment (scan sampled, paired samples t-test, n=30, p=0.365). From this it can be concluded that workers did not prefer one or the other brood. However, in the five worker experiment nine feedings were observed, all involving starved larvae. This suggests that workers were unable to distinguish between broods of starved and non-starved larvae from a distance, but that they were able to do so when they had access to the broods.

TABLE 1

First choice of workers that were alone or in a group of five and were given access to a brood of starved and a brood of non-starved larvae. First choice indicates the brood that was visited first.

Experiment	First Choice		χ^2	p-value
	starved brood	non-starved brood		
1 worker	12	13	0.040	0.841
5 worker group	24	23	0.021	0.884

Manually feeding a group of larvae in vivo

To study in vivo whether or not workers respond to the nutritional status of larvae and the corresponding strength of their hunger signal, in a colony six out of 12 queen larvae were selected and manually fed artificial food in order to saturate them. The other six larvae served as a control. The experiment lasted two hours, during which all feedings by workers were recorded.

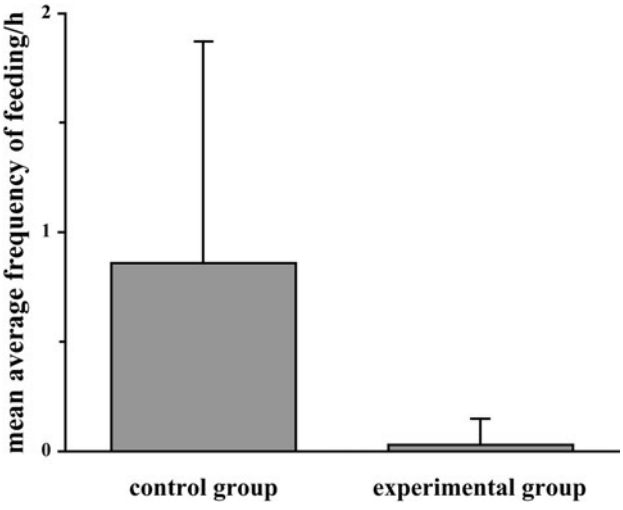

Fig. 2. – Mean average frequency/hour at which a control (n=18) and an experimental group of larvae (n=18) in a colony were fed by workers. Larvae in the experimental group were fed manually with artificial food. The frequency of feeding differs significantly between the two groups (t-test p=0.003).

Fig. 2 shows the mean average frequency at which larvae in the two groups were fed by workers during the experimental period. The control group was fed more by workers than the group that was fed manually (p=0.003). In fact, in the experimental group only one larva was fed once. Apparently, workers were able to perceive the nutritional status of larvae and they adjusted their feeding behaviour accordingly.

During the experiment a novel behaviour was observed: workers were seen to suck away the artificial food given to the larvae of the experimental group (mean frequency=2.17±1.61 times/hour/larvae, n=18). This "sucking away" occurred at least sometimes while larvae were still eating, which rules out the possibility that workers removed the artificial food because larvae were saturated.

Varying the number of larvae

In order to investigate the effect of the strength of the larval hunger signal on the feeding behaviour of individual workers, the pollen eating and feeding of workers confronted with broods of five or 15 starved larvae was observed. In addition, the data from the observations done in a colony were used (see before).

It was assumed that under the experimental condition a measure for the strength of the hunger signal, which triggers workers to feed larvae, is the duration of the adding pollen-first PE interval. Therefore the mean duration of this interval in the two experimental groups was compared. There was no difference between the five and 15 starved larvae groups (five starved larvae: mean 6271±5303s, 15 starved larvae: mean 4073±3529s, Mann Whitney U test, n=10 for both groups, p>0.10).

Another measure of the effect of the hunger signal on worker behaviour is the average frequency of feeding during the experimental sessions. This is shown in Fig. 3, in which the relationship between the average frequency of feeding in a session and the duration of the adding pollen-first PE interval for the two groups of workers is plotted. For both groups the correlation is not significant. However, an adding pollen-first PE interval longer than 4000s clearly corresponds with a low or zero frequency of feeding. The mean feeding frequency of the five starved larvae group tends to be higher than that of the 15 starved larvae group (Mann Whitney U test, p=0.081), contrary to the expectation. Interestingly, the average feeding frequency of a single worker in a colony is significantly higher than that in both experimental sessions (Kruskal Wallis p<0.05; Mann Whitney U test natural (n=29)-five starved larvae p=0.029, natural-15 starved larvae p<0.001, means±SD: natural 4.2±2.4, five starved larvae 2.3±2.1, 15 starved larvae 0.76±0.78 times/hour). This suggests that both experimental settings had an effect on worker feeding behaviour and indicates that the motivation of workers also plays a role.

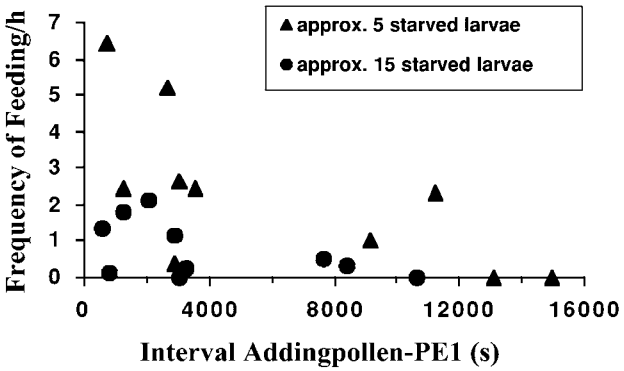

Fig. 3. – Feeding frequency after the adding pollen-PE1 interval versus the duration of the adding pollen-PE1 interval for the 5 (n=10) and the 15 (n=10) starved larvae group. For both groups there is no significant correlation (5 starved larvae r=-0.456, p=0.185, 15 starved larvae r=-0.398, p=0.255, combined (n=20) r=-0.236, p=0.317)).

Another measure of the strength of the hunger signal is the time between PE and the first feeding following that PE. A short PE-first feeding interval is assumed to reflect that a worker is reacting on the hunger signal. In Fig. 4 the mean duration of the PE-FE1 interval is shown for the three groups. The "natural" group does not differ from the five starved larvae group (Mann Whitney U test p=0.698) and both these groups have a shorter mean PE-FE1 interval than the 15 starved larvae group (Mann Whitney U test 15-natural p<0.001, 15-5 starved larvae p=0.001). Again, workers in the 15 starved larvae group appear to be less motivated by the hunger signal than workers in the five starved larvae group, contrary to the expectation. Thus, measuring the strength of the hunger signal is complicated by the effect of worker motivation.

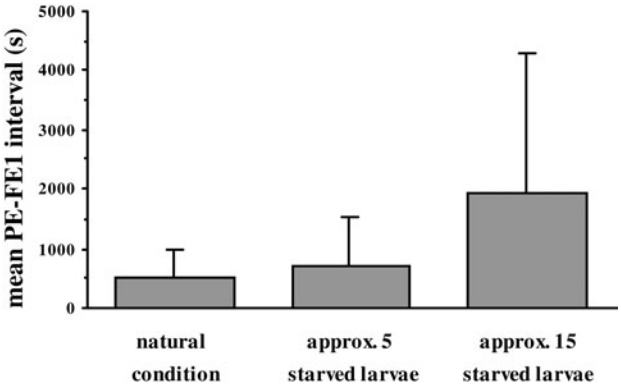

Fig. 4. – Mean time ±SD (s) between Pollen Eating (PE) and the following (first) feeding (FE1) for workers under natural condition (in a colony, n=48) and single workers confronted with 5 (n=45) and 15 (n=16) starved larvae. The mean duration of the PE-FE1 interval differs significantly between the 15 starved larvae group and the other two groups (Man Whitney U test 15 starved larvae-5 starved larvae p=0.001, 15 starved larvae-natural p<0.001, 5 starved larvae-natural p=0.698).

Fig. 5 shows that under a natural condition there is a low, but significant, negative correlation between the number of feedings and the PE-FE1 interval (r=-0.373, p=0.019), indicating that the duration of this interval is

indeed a possible measure of worker motivation. Interestingly, for the two experimental groups there is no significant correlation between the duration of the PE-FE1 interval and the number of feedings. This, once more, suggests that the experimental setting was too different from the natural condition, resulting in abnormal feeding behaviour of the workers.

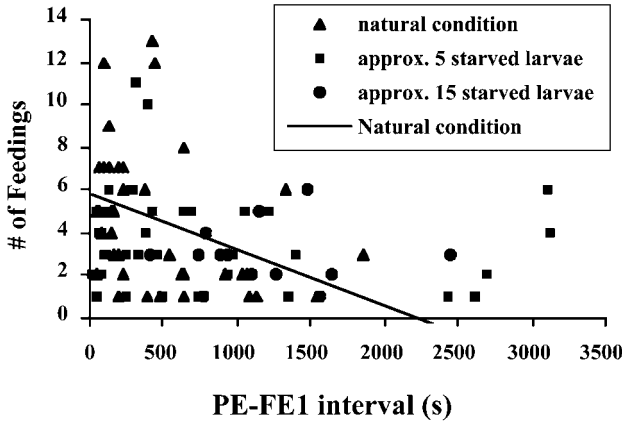

Fig. 5. – Number of feedings after Pollen Eating (PE) versus the time between PE and the first feeding for the three groups (n=39. n=39 and n=14 respectively). Only for the natural group there is a significant negative correlation between the PE-FE1 interval and the number of feedings after PE (r=-0.373, p=0.019. 5 starved larvae: r=-0.170, p=0.300, 15 starved larvae: r=0.195, p=0.504).

That worker motivation played a significant role in these experiments is further supported by the fact that there were big differences between the sessions of each group of experiments (using 5-15 starved larvae) in the amount of data obtained, due to differences in worker activity; some workers ate pollen and fed larvae a lot and seemed very "dedicated". Others kept on walking around and ate pollen and fed very little, seemingly not at ease and not paying as much attention to the larvae.

DISCUSSION

Feeding behaviour in general and the presence of a larval hunger signal in particular was studied in the bumble bee *Bombus terrestris*. We replicated the finding of Duchateau (unpublished data) that the duration of PE followed by feeding is on average longer than that of PE not followed by feeding. This in spite of the high degree of variation characterizing other aspects of bumble bee behaviour (Pendrel & Plowright, 1981; Pereboom, 1997; Ribeiro, 1997). We also found considerable variation in the duration and frequency of all aspects of feeding behaviour (standard deviations are usually close to the mean or even larger).

Results of the choice-experiment with a group of starved and a group of non-starved larvae show that workers are unable to perceive the difference in nutritional status between the larvae from a distance of about two centimeters. The fact that only starved larvae were fed shows that, after given the opportunity for closer examination, workers are able to distinguish between starved and non-starved larvae, as has previously been reported by Pereboom (1997). Furthermore, this supports the idea that larvae somehow advertise their nutritional status to workers by emitting some kind of hunger signal.

Further evidence that workers are able to perceive the nutritional status of larvae and adjust their behaviour accordingly was provided by the experiment in which a group of larvae was manually fed in vivo: feeding by the experimenters drastically decreased the rate of feeding by workers. On two of the three days experimentally fed larvae were not fed at all, and on one day one larva was fed only once. Interestingly, workers were observed to suck away artificial food from the larvae, also when they were still eating. A possible explanation for this behaviour is that too much artificial food was provided in one "feeding" and that consequently the excess was removed. Possibly, it was also to prevent dehydration of the larvae, which might result from the high osmotic value of the food.

Comparison of the feeding behaviour of workers confronted with broods of five and 15 starved larvae yielded some unexpected results. In the case of 15 starved larvae, workers were clearly less motivated to feed than in the case of five starved larvae: the average feeding frequency was lower and the mean PE-FE1 higher for the 15 starved larvae group. In the case of 15 starved larvae, the broods that were constructed often suffered from the increased mobility of hungry larvae, requiring the workers to repair the wax envelope. Sometimes larvae were pulled from the brood cup by workers and discarded. The wax envelopes of the five starved larvae broods usually were in better "shape". Comparison of the feeding behaviour of workers in a colony with that of workers in an experimental set up with a brood of five or 15 starved larvae yields the impression that on average in a colony workers receive a hunger signal approximately equal to or stronger than that of five starved larvae. The PE-FE1 interval and the number of feedings after PE were similar for the natural and the five starved larvae condition. However, for workers in a colony there was a significant negative correlation between the duration of the PE-FE1 interval and the number of feedings after PE. For both experimental groups this correlation was absent, indicating that not only the 15 starved larvae group but also the five starved larvae group gave rise to some extent to abnormal feeding behaviour. Therefore, it is likely that the experimental conditions of the five and 15 starved larvae were such that workers did not perform normal feeding behaviour. The results of these experiments suggest that the combination of the number of feedings after PE and the time between the end of that PE and the first feeding (PE-FE1) is a rough indicator of a worker's motivation to feed. A worker that feeds many times shortly after eating pollen is considered more

motivated than one that feeds only once, long after pollen eating.

On the whole, the data support the hypothesis that in *Bombus terrestris* larvae emit a signal allowing the workers to perceive their nutritional status. Furthermore, this signal can trigger a worker to eat pollen for a long time, and to subsequently feed larvae. That larvae actively solicit food in relation to their level of hunger has been reported in fire ants (CASSILL & TSCHINKEL, 1995, 1996, 1999a). There, larvae are even able to regulate their exact diet (CASSILL & TSCHINKEL, 1999b). Since in bumblebees all larvae basically receive the same food (PEREBOOM, 2000), it is unlikely that food soliciting by bumble bee larvae is as sophisticated as it is in fire ants.

Our results cast doubt on the hypothesis that workers impose a feeding regime on the larvae (RÖSELER, 1970; PLOWRIGHT & JAY, 1977). RÖSELER (1970) found that in *B. terrestris* caste is determined already in the first 3.5 days of larval development. With regard to caste differentiation he states that the queen pheromonally "instructs" the workers, who in turn regulate the rearing of the larvae into either workers or queens. RÖSELER (1991) elaborates on this by stating that last instar larvae respond to quantitative changes in nutrition (imposed by workers) by modulating their endocrine activity, which in turn triggers either the worker or the queen developmental pathway. Our results suggest that it is the larvae who, once determined to become either a worker or a queen, solicit food from the workers depending on their needs (among others related to their developmental stage).

The motivation of a worker also appears to play a role in the decision to feed (see e.g. the 15 starved larvae case). LINDAUER (1952) already suggested that bees (*Apis mellifera*), while patrolling in the nest, receive numerous signals, and on the basis of this information and their "Stimmung" ("mood", influenced by age and physiological state) devote themselves to a particular task. He adds that, in addition, signals of other bees could also influence their decision to feed or not. Furthermore, Lindauer reports that feeder bees inspect the larval cells, and he suggests that on the basis of the amount of food present in the cell or some other cue, they decide to feed or not. In bumble bees, as in bees, the division of tasks is also adapted to current colony needs (FREE, 1955). However, workers do not perform inspections in order to decide to feed larvae (no such behaviour was observed during this study, see also PEREBOOM, 1997; however see RIBEIRO, 1999). We suggest that workers perceive the nutritional status of larvae (by means of the larval hunger signal) during manipulation of the wax envelope that surrounds larvae.

From the above it follows that recruitment of workers to initiate feeding behaviour somehow needs to be regulated. It is plausible that workers have some threshold above which the larval hunger signal affects their behaviour, and that this threshold differs among workers depending on their physiological state, which in turn could depend on age, life history, food availability, temperature etc. (receptivity). If so, the signals of all larvae taken together will, through the effect they have on individual workers, eventually result in the regulation of feeding behaviour. In short, an individual worker needs to make an adaptive decision to go feeding or not depending on current larval and colony needs. The details of the interaction between larvae and workers and, more specifically, the effect of the larval hunger signal on worker behaviour require further research.

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