Contents

VOLUME 134 (Supplement 1)

ISSN: 0777-6276

Belgian Journal of Zoology

2004 134 (Supplement 1)

Belgian Journal of Zoology

AN INTERNATIONAL JOURNAL PUBLISHED BY
THE ROYAL BELGIAN SOCIETY FOR ZOOLOGY

Volume 134 (Supplement 1) – March 2004

PROCEEDINGS OF THE 9th BENELUX CONGRESS OF ZOOLOGY
"ADAPTATION & CONSTRAINT"

THE BELGIAN JOURNAL OF ZOOLOGY IS COVERED IN CURRENT CONTENTS, SCIENCE CITATION INDEX AND IN OTHER LEADING REFERENCE PUBLICATIONS

BELGIAN JOURNAL OF ZOOLOGY

Belgian Journal of Zoology

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The Belgian Journal of Zoology is published with the financial support of the "Direction générale de l'Enseignement supérieur et de la Recherche scientifique" and of the "Universitaire Stichting van België/Fondation Universitaire de Belgique".

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Belgian Journal of Zoology

Published by the
"KONINKLIJKE BELGISCHE VERENIGING VOOR DIERKUNDE —
SOCIÉTÉ ROYALE ZOOLOGIQUE DE BELGIQUE"

PROCEEDINGS OF THE 9th BENELUX CONGRESS OF ZOOLOGY "ADAPTATION & CONSTRAINT"

Editors:

Hans Van Dyck,
Marcel Eens
and Peter Aerts

Volume 134 (Supplement 1)
(March, 2004)

Managing Editor of the Journal :

Ernest Schockaert
Department SBG
Limburgs Universitair Centrum
B-3590 Diepenbeek (Belgium)

Printed in Belgium (February 2004) by
Drukkerij George Michiels, N.V., Tongeren

Belg. J. Zool., 134 (supplement 1) : 3-7 March 2004

Colour variation and crypsis in relation to habitat selection in the males of the crab spider *Xysticus sabulosus* (Hahn, 1832) (Araneae : Thomisidae)

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ABSTRACT. The crab spider *Xysticus sabulosus* (Hahn, 1832) is a sit-and-wait predator, typical for sandy habitats in Europe and Flanders. In the Flemish coastal dunes, the species is very abundant in grey and blond dunes. Its abdominal and cephalothorical colour varies from almost completely white to dark brown. During autumn 2001, we investigated crypsis as a function of the occupied micro- and macrohabitat. Our results indicate that (1) Colouration differs between the populations. The species is darker in a grey dune completely covered with dried (brown) mosses and in a humid dune slack covered with algae, than in populations from a sea inlet and from a blond dune. The colouration of a population from a grey dune with mosses, lichens and bare sand is intermediate. (2) Individual cephalothoracal colouration is, in contrast to abdominal colouration, related to microhabitat selection : individuals with darker cephalothoraxes occupy hunting sites with a higher coverage of mosses, while those with a pale one are found in microhabitats with a high amount of nude sand. The observed spider colour-environment covariation between populations is probably the result of natural selection or colour alteration during the juvenile development. Further research on these possible underlying mechanisms remains, however, necessary.

KEY WORDS : Coastal dunes, microhabitat, foraging, predation.

INTRODUCTION

Colour variation within the same species is a characteristic of many invertebrates. Especially in molluscs, butterflies, grasshoppers and spiders, this phenomenon has been studied within the framework of apostatic selection (Allen, 1988, Endler 1988, 1990), sexual selection (Hausmann et al., 2003), thermoregulation (Whitman, 1988; Fosman, 2000) and crypsis (Endler, 1981, 1984, 1988).

According to Endler (1991), colour pattern is cryptic if it resembles a random sample of the visual background as received by its predator at the time and place at which the prey is most vulnerable to predation. In the case of predators, crypsis should, hence, also occur in the situation where predation efficiency strongly depends on the visual perception of prey. This colour matching in relation to the substrate can result from a rapid, physiological response or from a slow, morphological adaptation (Moreteau, 1975), more specific from ontogenetic colour adaptation (Graf & Nentwig, 2001), sometimes induced by the diet (Schmalhofer, 2000) or by natural selection in the case of colour pattern inheritance (Endler, 1983). There is a general consensus that crypsis increases the species' survival chances by reducing its own predation rate (i.g. Stimson, & Bergman, 1990; Endler, 1991; Götmark, 1994; Kingsolver, 1996; Forsman & Appelqvist, 1999). Documented studies on benefits resulting from an increased foraging success are, however, rare. In the case of the latter, foraging and reproductive success are highest if the animal colour pattern matches the colour of the substrate (Fritz & Morse, 1985). In a web-building spider, ontogenetic colouration changes were related to changes in web-structure, presumably as an adaptation to reduce intraspecific competition (Graf & Nentwig, 2001).

In spatial heterogeneous environments, colour polymorphism induces the use of different microhabitats, as demonstrated for grasshoppers in mosaic grasslands (Calver & Bradley, 1991), for juvenile crab spiders and flower inflorescence selection (Schmalhofer, 2000), and for marine isopods in macro-algal vegetation (Merilaita & Jormalainen, 1997).

Our study animal, the crab spider *Xysticus sabulosus* (Hahn, 1832) is a ground-active sit-and-wait predator, typical for sandy habitats in Europe and Flanders (Maelfait et al., 1998; Roberts, 1995). In the Flemish coastal dunes the species is abundant in grey and blond dunes (Bonte et al., 2002). Males are active in autumn, while females are characterised by an activity peak in autumn and spring. Its abdominal and cephalothoracal colouration varies from almost completely white to dark brown, and is constant during the adult phase (Bonte, pers. obs.). Ontogenetic changes in colouration during juvenile development have not yet been investigated.

In this contribution we investigate how spider colouration varies between several populations and if colouration is adapted to the habitat (i.e. substrate). We hypothesise that in sandy habitats (Marram dunes, sea inlet), *X. sabu-*

losus should be coloured paler than in grey dunes with a heterogeneous moss, lichen and sand coverage, and that colouration should be darkest in dune vegetation dominated by mosses, which colour brown during dry or dark green during wet weather conditions. Additionally, we investigate if microhabitat selection in the heterogeneous grey dune is induced by the spider colouration. By comparing relations between the abdominal and cephalothoracal colouration and the patterns of microhabitat selection, potential crypsis-inducing patterns are investigated. We especially question whether crypsis is the result of a predator-avoiding strategy or if optimal foraging induces it.

MATERIAL AND METHODS

Study area and sampling methodology

Five populations were sampled in the coastal dune complex of De Panne, Bray-Dunes and Ghyvelde, at the French-Belgian Border. The sampled habitats differed in soil- and vegetation structure, ranging from completely nude sandy habitats (Marram dunes, sea inlet), through moss- and lichen grey dune with a substantial amount of bare sand, to completely moss (*Campylopus intraflexus*)-dominated dunes and a dune slack covered with a dark algae layer. This habitat variation corresponds with the colouration of the substrate : pale white to grey coloured in the case of the first two habitats dominated by bare sand, intermediately (grey-brown) coloured in the case of the grey dune with *Cladonia* lichens and dried mosses (*Tortula ruralis* and *Hypnum cupressiforme*) and dark grey to black coloured in the case of the *Campylopus* vegetation and the dune slack.

In each habitat we sampled spiders during September 2001, with five pitfall traps (diameter 9 cm, filled with a formalin-soap solution). A total of 136 males were collected in the five sampled habitats : 22 in the Marram dune, 21 in the Sea inlet, 24 in the dune slack, 38 in the lichen-grey dune and 31 in the *Campylopus*-dominated grey dune.

In a second experiment, 100 small pitfalls (diameters 3 cm, same fixative, distance between traps : 30 cm) were placed in a grid in one habitat, the varied grey dune. Around each trap, the coverage of bare sand, lichens and dried mosses was estimated. The latter set-up was used to study the possible effect of colouration on the microhabitat selection. Here, 174 individuals were sampled.

Colour determination

Dorsal digital photographs of each specimen were taken with a high quality digital camera (2800000 pixels), placed on a binocular microscope with magnification 10x, under standardized lighting conditions. The mean pixel value in the Red-Green-Blue (RGB channels) spectrum was determined by using the software Corel Photo-Paint, version 7.373. The brightness of each pixel was determined and averaged for, respectively, the abdominal and cephalothoracal parts. Pixel values range from 0 (black) to 255 (white). Colouration of the macro- and microhabitat was determined in the same way from, respectively, digital aerial photographs (taken from an aeroplane, flying 50 meters above the habitats) and digital photographs taken from 1-meter height above the surface. Photographs were taken on the same day within a short time span, so light conditions were similar. Macro-habitat substrate colouration values were (means ± SD) 203.11 ± 38.57 for the Marram dune, 217.50 ± 13.38 for the sea-inlet, 103.45 ± 15.70 for the dune slack, 188.15 ± 28.27 for the lichen-grey dune and 95.38 ± 4.66 for the *Campylopus*-dominated grey dune. Since both habitat and spider colouration vary within the range white-grey-brown-black, mean values of the RGB channels are a good quantification of the substrate and animal colour.

Data analysis

General results. The relationship between cephalothoracal and abdominal colouration was investigated by Pearson correlation since all spider colouration data were normally distributed. Principal component analysis was applied for the detection of covariances between the estimated coverage of mosses, lichens and bare sand. The relationship between component scores (one significant axis; see results) and microhabitat colour were again analysed with Pearson correlation.

Colour variation between the sampled populations. Only colouration data from males were used, since only eleven females were caught during the sampling period. Since variances were unequal, a Kruskal-Wallis ANOVA was applied for the analysis of colour variation between the five populations (habitats). Mann-Whitney U-tests, Bonferroni-corrected for multiple testing (P-level of 0.0041), were used as post-hoc tests for the determination of colour differences between the specific habitats.

Colour variation and microhabitat-selection. Spearman rank tests were used for the analysis of microhabitat selection as a function of component scores, related to the coverage of bare sand (very pale substrate), lichens (grey substrate) and dried *Tortula* mosses (dark brown substrate). P-values were again Bonferroni-corrected ($P<0.025$).

Analyses were performed with Statistica 5.5 (Statsoft 2000).

RESULTS

General results

Colouration values of the cephalothorax ranged from 54.32–137.36, with a mean of 99.787; those from the abdomen ranged between 50.23–157.84, with a mean value of 91.66. Both values are significantly correlated ($r_{309}=0.102$; $P<0.0001$). The colouration was in general analogous for the cephalothorax and the abdomen, but slightly darker on the latter. Three examples of spiders with different colouration are given in Fig. 1.

The proportional coverage by *Tortula* mosses, *Cladonia* lichens and bare sand was related to one principal component. Component loadings (r_{99}) were -0.801 for moss, -0.668 for lichen and 0.999 for sand coverage. Component scores hence reflect the transition from moss to bare sand dominance and are correlated with the microhabitat colouration ($r_{35}=0.268$; $P<0.001$). The moss, lichen and sand coverage range gives thus a good reflection of the microhabitat colouration.

Fig. 1. – A light (left), intermediate (middle) and dark (right) coloured individual *Xysticus sabulosus.* Carapax colouration scores (mean ± SD) : 136.44 ± 37.89, 95.33 ± 35.13 and 60.38 ± 37.74, respectively.

Colour variation between the sampled populations

The mean values of both the cephalothoracal and abdominal colouration, with 95% confidence intervals are visualised in Fig. 2. Results of the Kruskal-Wallis ANOVA revealed significant inter-population variation for the cephalothoracal (Kruskal-Wallis test : $H(4, N=136)=68.012$; $P<0.0001$) and the abdominal colouration (Kruskal-Wallis test : $H(4, N=136)=12.719$; $P=0.0127$). Bonferroni-corrected post-hoc Mann-Whitney U-tests only indicate significant differences in the abdominal colouration between the population from Marram dunes and the one from the *Campylopus*-dominated grey dune ($P=0.002$). Differences between populations (habitats) were more pronounced for the colouration of the cephalothorax : only the population from the Sea inlet did not differ from those from Marram dunes ($P>0.05$) or from the sandy, lichen-rich grey dune ($P=0,006$, NS after Bonferroni-correction).

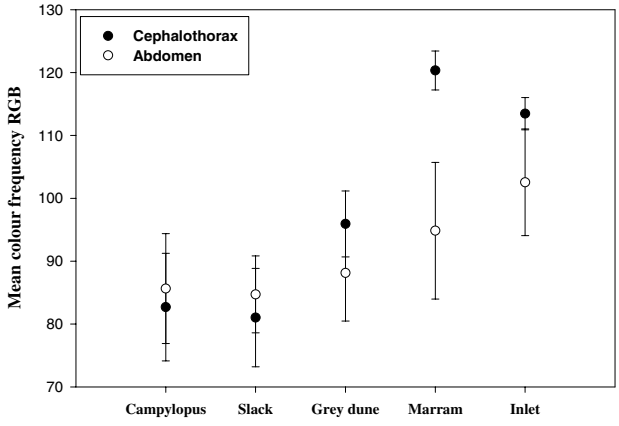

Fig. 2. – Variation in spider colouration between the five populations (mean values and 95% confidence intervals). Legend : *Inlet* : Sea inlet; *Marram* : Marram dune; *Grey dune* : heterogeneous moss-sand-dominated dune; *Slack* : pioneer dune slack; *Campylopus* : dark mosses-dominated grey dune.

Colour variation and microhabitat-selection

Correlation of the abdominal and cephalothoracal colour with the substrate principal components revealed significant results for both ($R_{abd,173}=0.159$; $P=0.036$; $R_{Ceph,173}=0.217$, $P=0.04$: Fig. 3). The relationship between abdominal colouration and the substrate component is, however, not significant after Bonferroni-correction.

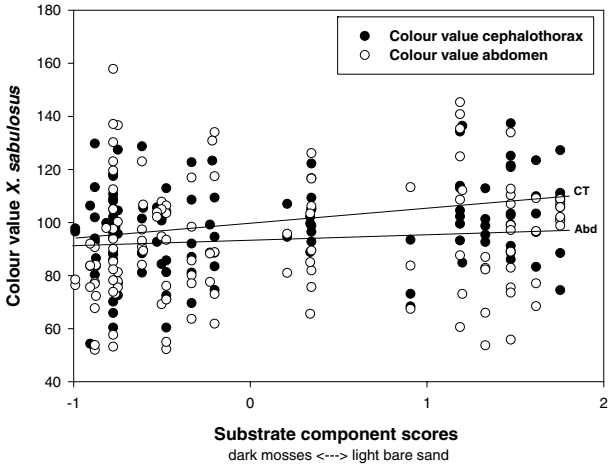

Fig. 3. – Relationship between substrate colouration (as revealed by substrate component scores) and spider colouration (cephalothorax and abdomen).

DISCUSSION

Body colouration of the crab spider *X. sabulosus* gradually varies between whitish and dark brown, with a high frequency of intermediate-coloured individuals. Cephalothoracal and abdominal colouration covary significantly, although pale individuals with dark abdomen and vice versa do occur. Crypsis was observed for both body parts between macrohabitats, but was only significant for the cephalothoracal colouration in the case of microhabitat selection.

Colouration of the cephalothorax and abdomen showed distinct differences between the sampled populations, although only for the first could significant differences be detected as a function of the habitat : spiders with a dark cephalothorax were significantly more abundant in the dune slacks and the moss dune dominated by *Campylopus intraflexus*. Intermediate-coloured spiders were found in the heterogeneous grey dune, while pale individuals occurred in the dune habitats, dominated by nude sand. Crypsis was, therefore, detected between spider and substrate colouration. Interdemic variation in colouration can result from natural selection (Endler, 1983) or from ontogenetical morphological adaptations (Moreteau, 1975; Graf & Nentwig, 2001). Unfortunately, the possible underlying genetic mechanisms have not yet been studied in this species. Studies on colour variation in another crab spider indicate that morphological colour changes are induced by the diet (Schmalhofer, 2000)

and the substrate during juvenile development (HOLL, 1987). This mechanism for colouration changes during juvenile development in a related crab spider allows us to assume that natural selection during the juvenile development may be an explanation for the observed colour differentiation, although only during the early developmental stages, in which spider colouration is not yet adapted to the substrate.

In the adult phase, colouration is fixed (BONTE, pers. observ.), and in spatially heterogeneous environments is responsible for the observed related microhabitat selection. Selection of suitable microhabitats for crypsis may be induced by one or more of three possible mechanisms (which also underlie natural selection) : avoiding predation, increasing foraging success (which is directly related to fitness) or optimal thermoregulation (ENDLER, 1991). The temperature on moss is only significantly higher than on bare sand during warm summer days. During colder days (the period in which our species is adult and active), temperatures do not differ between these locations (GHESQUIERE, pers. comm.). Crypsis will hence not result from thermoregulatory processes since substrate choice would not enable certain colour forms to heat up faster than others.

Avoidance of predation is the second possible reason for the observed crypsis. This implies that predators should be visually orientated. Crab spiders are however sit-and-wait predators with a low cursorial activity and are slightly conspicuous for potential predators. Although scarcely documented, Pompililidae and Ichneumonoidae wasps (FITTON et al., 1987), which detect prey possibly by olfactory stimuli (as detected for the parasitoid *Gelis festinans*, VAN BAARLEN et al., 1996) can be regarded as their most important predators in coastal dune ecosystems (NOORDAM, 1998). Since abdominal crypsis is less pronounced, avoidance of predation is probably of minor importance for the elucidation of the observed crypsis. By contrast, both the passive hunting strategy and the highly significant covariation between substrate and cephalothorax colouration indicate that crypsis is related to optimal foraging. Since prey have to approach the crab spider as closely as possible, an optimally-camouflaged cephalothorax should be beneficial for prey capturing. In heterogenous environments, such as the investigated grey dune with lichens, mosses and bare sand, individuals are, on average, intermediately coloured, but considerable intraspecific variation occurs. This variation results in differential microhabitat use, dependant of the colouration. Possibly, this microhabitat selection results from previous experience or from certain inherited behavioural responses, in which the spider is able to link its own body colouration to the selection of suitable hunting sites.

Crypsis is thus obvious in populations of the crab spider *X. sabulosus* in the Flemish coastal dunes and linked to microhabitat selection in a spatially-heterogeneous grey dune. The underlying evolutionary mechanisms for the observed interdemic variation (the result of natural selection or from ontogenetic changes?) and the observed relationship to microhabitat selection (inherited behavioural response or the result of earlier experience?) remain unclear and need to be elicited in future research.

REFERENCES

ALLEN, J.A. (1988). Frequency-dependent selection by predators. *Phil. Trans. Roy. Soc.* B., 319 : 485-503.

BONTE, D., L. BAERT & J.-P. MAELFAIT (2002). Spider assemblage structure and stability in a heterogeneous coastal dune system (Belgium). *J. Arachnol.,* 30 : 331-343.

CALVER, M.S. & J.S. BRADLEY (1991). A microhabitat selection explain sex-related colour morph frequencies in the grasshopper *Acrida conica* Fabricius? *Anim. Behav.,* 41 : 1101-1102.

ENDLER, J.A. (1981). Natural and sexual selection on colour patterns in poeciliid fishes. *Envir. Biol. Fishes,* 9 : 173-190.

ENDLER, J.A. (1983). An overview of the relationships between mimicry and crypsis. *Biol. J. Linn. Soc.,* 16 : 25-31.

ENDLER, J.A. (1984). Progressive background matching in moths and a quantitative measure of crypsis. *Biol. J. Linn. Soc.*, 22 : 187-231.

ENDLER, J.A. (1988). Frequency-dependent predation, crypsis and aposematic colouration. *Phil. Trans. R. Soc.* B, 319 : 505-523.

ENDLER, J.A. (1990). On the measurement and classification of colour in studies of animal colour patterns. *Biol. J. Linn. Soc.*, 41 : 315-352.

ENDLER, J.A. (1991). Interactions between predator and prey. In : *Behavioural Ecology* (3rd edn). KREBS, J.R. & N.B. DAVIES (Eds.). Blackwell, Oxford : 169-196.

FITTON, M.G., M.R. SHAW & A.D. AUSTIN (1987). The Hymenoptera associated with spiders in Europe. *Zool. J. Lin. Soc*., 90 : 65-93.

FORSMAN, A. & S. APPELQVIST (1999). Experimental manipulation reveals differential effects of colour pattern on survival in male and female pygmy grasshoppers. *J. Evol. Biol.,* 12 : 391-401.

FRITZ, R.S. & D.H. MORSE (1985). Reproductive success and foraging of the crab spider *Misumena vatia*. *Oecologia,* 65 : 194-200.

GÖTMARK, F. (1994). Does a bright colour patch increase or decrease predation? Red wings reduce predation risk in European black birds. *Proc. R. Soc. Lond. B*, 256 : 83-87.

GRAF, B. & W. NENTWIG (2001). Ontogenetic change in coloration and web-building in the tropical spider *Eriophora fuliginea* (Araneae, Araneidae*). J. Arachnol*., 29 : 104-110.

HAUSMANN, F., K.E. ARNOLD, N.J. MARSHALL & P.F. OWENS (2003). Ultraviolet signals in birds are special. *Proc. R. Soc. Lond. B*., 270(1510) : 61-67.

HOLL, A. (1987). Coloration and chromes. In : *Ecophysiology of spiders*. NENTWIG W. (ed.). Springer-Verlag, Berlin : 16-25.

KINGSOLVER, J.G. (1996). Experimental manipulation of wing pigment pattern and survival in western white butterflies. *Am. Nat.,* 147 : 296-306.

MAELFAIT, J.-P, L. BAERT, M. ALDERWEIRELDT & M. JANNSENS (1998). A Red list for the spiders of Flanders. *Bull. K. Belg. Inst. Natuurwet., Entomologie,* 68 : 131-142.

MERILAITA, S. & V. JORMALAINEN (1997). Evolution of sex differences in microhabitat choice and colour polymorphism in *Idotea baltica. Anim. Behav.,* 54 : 769-778.

MORETEAU, B. (1975). Fonction chromatrope de la pars intercerebralis chez l'acridien *Oedipoda caerulescens. J. Insect Physiol.,* 21 : 1407-1413.

NOORDAM, A. (1998). De strijd van duinspinnen met mieren en wespen. *Duin,* 21(3), 4-6.

ROBERTS, M. (1995). *Spinnengids*. B.V. Uitgeversmaatschappij Tirion, Baarn (397 pp).

SCHMALHOFER, V.R. (2000). Diet-induced and morphological color changes in juvenile crab spiders (Araneae, Thomisidae). *J. Archnol*., 28 : 56-60.

STIMSON, J. & M. BERGMAN (1990). Predator induced color polymorphism in *Daunaus plexippus* L. (Lepidoptera : Nyphalidae) in Hawaii (USA). *Heredity,* 65 : 401-406.

VAN BAARLEN, C.J. TOPPING & K.D. SUNDERLAND (1996). Host location by *Gelis festinans*, an eggsac parasitoid of the linyphiid spider *Erigone atra*. *Entomol. Exp. Appl.*, 81 : 155-163.

WHITMAN, D.W. (1988). Function and evolution of thermoregulation in the desert grasshopper *Taeniopoda eques*. *J. Anim. Ecol*., 36 : 363-373.

Belg. J. Zool., 134 (supplement 1) : 9-13 March 2004

Morphological and physiological differences between mummy colour morphs of *Aphidius rhopalosiphi* (Hymenoptera : Braconidae) : an adaptation to overwintering?

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ABSTRACT. Colours of individuals are one of the more current morphological clues linked to overwintering adaptations. We studied morphological and physiological differences between colour morphs of *Aphidius rhopalosiphi* mummies in relation to possible adaptation to winter survival. Aphids *Sitobion avenae* were parasitised and placed in a controlled temperature room at 10˚C, under a photoperiod of L9 :D15. Mummies were sorted by colour. Volume, fresh mass, water content, development time and cocoon thickness were recorded individually. Differences in development time were observed between dark and white forms, cocoon thickness and water content. We could not relate these difference to diapause status. However, these features are very likely linked to distinct cold resistance patterns and may be indications of a quiescence state.

KEY WORDS : *Aphidius rhopalosiphi*, morph colour, water content, development time, cocoon thickness, diapause, quiescence, cold resistance.

INTRODUCTION

In temperate climates, many insects spend an important part of their lives in an overwintering stage. These insects show a large range of adaptive responses to the detrimental winter conditions. Moreover, within populations different strategies may be present and individual variation reinforces the adaptive range. For instance, several species exhibit a large variability in the colour morphs present within winter populations (Leather et al., 1993). Colour could play an important role in thermoregulation. According to Schlinger & Hall (1959) the white-coloured cocoon of *Trioxys utilis* Muesebeck, 1956 (Hymenoptera : Braconidae) is more common in warmer weather – white colour acting as heat reflectant – whereas dark brown cocoons are mostly encountered under cold weather conditions where the colour contributes to more efficient heat absorption. Dark colour is also an advantage to those insects that overwinter as immobile, concealed forms, as cryptic colour offers some protection against predators that depend on vision (Leather et al., 1993). Some authors have stated that, for parasitoids, colour of mummies may be an efficient and practical tool for distinguishing between diapausing and non-diapausing individuals. Schlinger & Hall (1960) have indeed shown that diapausing individuals of *T. utilis* exhibited a dark brown colour and a much thicker cocoon. Previous works suggested that diapausing and non-diapausing individuals of *A. rhopalosiphi* (De Stefani-Peres) can be safely recognized by the colours of their mummies (Krespi et al., 1994; Langer & Hance, 2000; Rigaux et al., 2000). Krespi et al. (1994) noted that diapausing last instars of *A. rhopalosiphi* formed darker mummies and thicker cocoons than those of non-diapausing parasitoids. Similar differences have been observed in a number of Aphidiids by Stàry (1970).

During a three generations experiment, Langer & Hance (2000) induced diapause in *A. rhopalosiphi* individuals. In accordance with the literature, they assigned black mummies to diapausing ones and stated that, after three generations, some of the mummies remained white and thus non-diapausing. As mild winters are quite frequent in Belgium, they concluded that *Aphidius rhopalosiphi* (De Stefani-Peres) (Hymenoptera : Braconidae : Aphidiinae) may overwinter using two different strategies : diapause and quiescence of the last instar larvae within the aphid mummies. However, in nature and in laboratory experiments, we observed a large range of *A. rhopalosiphi* mummy colours whatever the conditions. Our aim was thus to study morphological and physiological differences between colour morphs of *A. rhopalosiphi* mummies, formed under the same constrained climatic conditions, in order to better understand the role of colour variations for this species.

MATERIAL AND METHODS

Laboratory cultures of *A. rhopalosiphi* and its host *Sitobion avenae* Fabricius 1775 (Homoptera : Aphididae) were started from individuals collected in winter wheat fields during summer 1999 at Louvain-la-Neuve, Belgium (50.3˚N latitude). Aphids and parasitoids were reared on

winter wheat seedlings, *Triticum aestivum* (L.), at 20˚C under a photoperiodic regime of L16 :D8. Experiments were carried out from March 2000 until March 2001. A batch of *ca*. 5000 second and third instars of *S. avenae* was exposed during 48 hours to 50 pairs of parasitoids *A. rhopalosiphi*. Aphids were then placed in a controlled temperature room at 10˚C under a photoperiod of L9 :D15, corresponding to autumnal conditions in Belgium (Rigaux et al., 2000). Once mummies were formed at 10˚C, *i.e.* 25 days after exposure, they were collected and separated according to colour. To do that, we removed individuals from the control room at the same time, the first day the first mummies were noticed. Then, they were classified into three groups of *ca*. 100 individuals each, using a universal RAL colour chart. We considered that colours RAL 1001, RAL 1002, RAL 1013, RAL 1014, RAL 1015 and RAL 7044 represented white mummies (n=101), whereas brown mummies (n=95) were characterized by RAL 8008, RAL 8011, RAL 8024, RAL 8025 and RAL 8028. For this study, we did not take into account the intermediate colours RAL 1004, RAL 1011, RAL 1019, RAL 7006 and RAL 7008. For each specimen, we recorded volume, dry mass, water mass, water content, development time and cocoon thickness. A colour video module connected to a monitor and a micrometric scale was used to determine the size of each mummy, total length (L) and width (Wi). Volume was calculated using the formula (πWi*Wi*L)*(4/3). Fresh mass was recorded for each individual with an electro balance with a precision of 1 µg (Mettler Me22). Some of these mummies were used to measure individual dry mass and to calculate water mass and water content. The other mummies were left in the same conditions until emergence in order to record the time to emergence and cocoon thickness.

Development time and thickness of the cocoon

Thirty-six dark and thirty-eight white mummies were reared until emergence at 20˚C, L9 :D15. The development time is the total duration between egg laying and adult emergence recorded individually at 12-hour intervals. Development time on a day-degree (˚D) basis was computed as : $^{\circ}D=DT\ (T-T_o)$ for $T > T_o$, where T is the temperature in ˚C, DT is the observed developmental period in days and T_o the lower thermal threshold. For *A. rhopalosiphi* we used 6˚C for the thermal threshold (Campbell et al., 1974; Ruggle & Holst, 1994; Sigsgaard, 2000). The thickness of the cocoon wall was measured for 12 dark mummies (three repetitions per mummy) and 10 white mummies (three repetitions per mummy) by scanning with an electron microscope (JSM 6301F).

Dry mass, water mass and water content

The dry masses of 63 white and 59 dark mummies were obtained after drying for three days in an oven at 60˚C (Vernon & Vannier, 1996; Worland et al., 1998). A desiccator was used to transfer each mummy from the oven to the balance. Mummies remained out of the dry air of the desiccator for less than five minutes and each specimen was placed into an aluminum curl paper to prevent damage. Water mass (Wm) was individually calculated as the difference between the fresh mass (Fm) and dry mass (Dm). The fresh water content, "Wf" is the total water mass expressed as a percentage of the fresh mass. The dry water content "Wd" is the total water mass expressed as a percentage of the dry mass (Chauvin & Vannier 1997).

Statistical analysis

Normality of the data was assessed for thickness of the cocoon, water mass expressed as a percentage of fresh mass and as a percentage of dry mass. Logarithmic transformation (ln) and normality were assessed for volume, fresh mass, dry mass and water mass. Development time data were non-normally distributed. Analysis of variance (ANOVA) and Kruskal-Wallis test were used to determine the significance of observed differences between the two colour groups. The analyses were performed using Statistical Analysis System (SAS Institute, 1990).

RESULTS

All results (mean ± SEM) are summarized in Table 1. No differences were found between dark and white forms mass of *A. rhopalosiphi* mummies in volume, fresh mass, dry mass or water ($p > 0.05$).

In our experiment, the time between egg and mummy stage (at 10˚C) was 25 days. The elapsed time between the mummy stage and adult emergence was 3.5 days for the first emergence and 10 days for the last. Mean development time of dark mummies was longer than that observed for white ones (mean difference of 13.39 ˚D, $p < 0.05$, Table 1). This difference is obviously too low to

TABLE 1

Comparison between dark and white mummies for each criterion and the level of significance between colours. Time before emergence was non-normally distributed and the non-parametric Kruskal-Wallis test has been used in that case.

Variable	N		Means ± SEM		Analysis
	Dark	White	Dark	White	
Volume (mm^3)	95	101	8.71 ± 0.26	8.64 ± 0.27	F=0.18 P=0.6741 NS
Fresh mass (mg)	95	101	0.43 ± 0.01	0.41 ± 0.01	F=0.34 P=0.5632 NS
Dry mass (mg)	59	63	0.16 ± 0.01	0.16 ± 0.01	F=0.00 P=0.9614 NS
Water mass (mg)	59	63	0.26 ± 0.01	0.29 ± 0.01	F= 2.91 P=0.0908 NS
Water content/Dry mass (%)	59	63	165.08 ± 2.87	182.27 ± 2.84	F=18.09 P<0.0001 ***
Water content/Fresh mass (%)	59	63	62.02 ± 0.42	64.35 ± 0.37	F=17.71 P<0.0001 ***
Mummies thickness (µm)	12	10	16.47 ± 1.45	12.10 ± 1.28	F=4.88 P=0.0390 *
Time before emergence (° days)	36	38	182.44 ± 4.46 (156-240)	169.05 ± 2.76 (149-212)	$\chi 2$=3.86 P=0.0493 *

conclude that brown mummies were in diapause. Values for white mummies ranged between 149 and 212˚D, whereas for dark ones they ranged between 156 and 240˚D. A great overlap between colour morphs was thus obvious (Fig. 1), and both dark and white mummies could have a short development time.

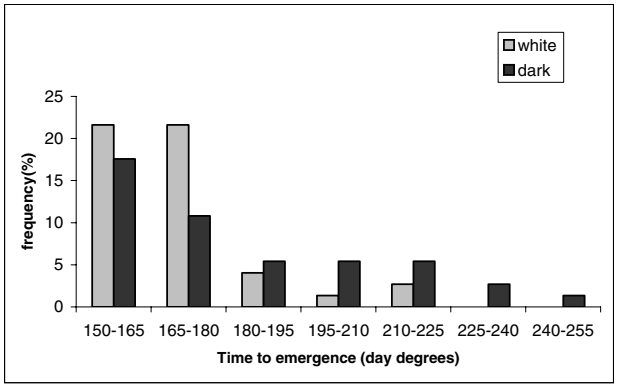


Fig. 1. – Frequency distribution for dark and white mummies, in relation to time before emergence.

Concerning the thickness of the cocoon, dark mummies were very significantly thicker than white ones ($p < 0.0001$), even though values overlapped (Fig. 2). The thinnest dark mummy had a wall of 10.00 μm, while the minimum for white mummies was 2.78 μm. The thickest mummy was 26.15 μm for dark mummies and 20.51 μm for white ones.

A highly significant difference between the two colour morphs was recorded for water content expressed both as a percentage of the dry mass or as a percentage of the fresh mass ($p < 0,0001$). In both cases, the mean water content was lower for dark mummies than for white ones (Table 1). Figure 3 shows frequency distribution of water content (Wd) as a function of the colour morph. Even though dark mummies exhibited lower water contents than white ones, the distribution of water contents overlapped widely.

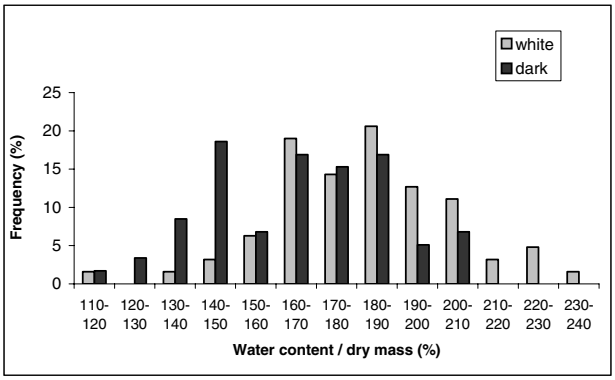
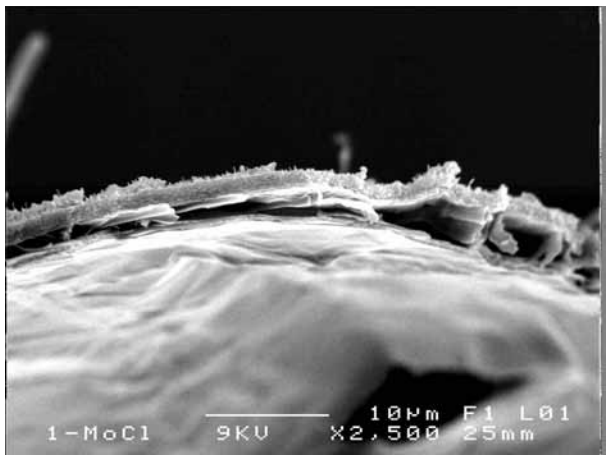


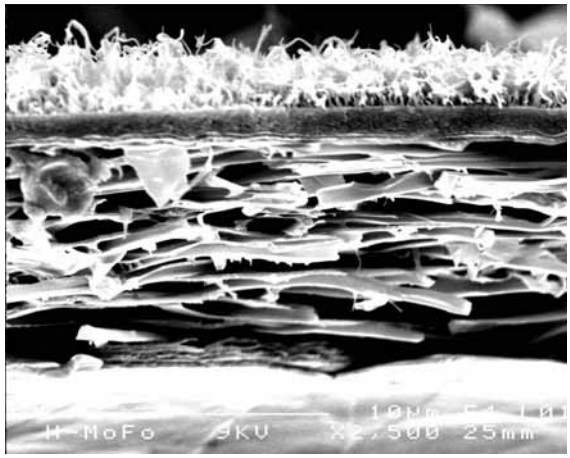
Fig. 3. – Frequency distribution of water content, in relation to dry mass, for dark and white mummies.

DISCUSSION AND CONCLUSIONS

Ruggle & Holst (1994) considered that the current development time for non-diapausing *A. rhopalosiphi*



1) White mummy : 3.69 μm



2) Dark mummy : 20.77 μm

Fig. 2. – Cross-section through the mummy (cocoon of the parasitoid and aphid cuticle) of one white and one dark mummies (pictures 1 and 2).

individuals is 194˚D and Sigsgaard (2000) considered it to be 205˚D. Krespi et al. (1997) observed that most adults of *A. rhopalosiphi* emerged within a month following oviposition and required 200-360˚D to complete development. However, in their case, 0.7% of parasitoids needed 480-560 ˚D and 1.5% required 1140 to 1580˚D. Regarding these high values, Krespi et al. (1997) underlined that these individuals were probably in diapause. In our experiment none of the mummies took such a long time for development, indicating that even the dark mummies we obtained were not in diapause. Indeed, the longest development time recorded was only 240˚D, well below the value given by Krespi et al. (1997) for diapausing individuals. It is still possible that some dark mummies with a longer development time were in quiescence. Indeed, this type of dormancy is probably a phenomenon confined to early winter or to winter-active insects, and only results in growth retardation (Leather et al., 1993).

Considering the cocoon thickness, Krespi et al. (1997) observed similar values to us with thickness less than 10 μm for non-diapausing individuals of *A. rhopalosiphi* and between 20 and 40 μm for diapausing ones. In their case as in ours, only yellowish and brownish mummies, and not intermediate colours, were taken into account. In our

experiment, some of the dark brownish mummies also had a thick cocoon, but were not diapausing. In a study on *Aphidius smithi* (Sharma et Subba Rae) (Hymenoptera : Braconidae), WIACKOWSKI (1962) observed three kinds of cocoon : white-brown and thin, dark-brown and thin, and dark-brown very thick, the last one corresponding to a typical diapause. We must further investigate the possibility of similar trends in *A. rhopalosiphi* mummies. One principal role of the silken cocoon is probably to protect the parasitoid from desiccation. In some parasitoids the overwintering cocoons (low ambient humidity) are more robust than summer ones (high ambient humidity) (TAGAWA, 1996). Within our data, it is possible that dark morphs were cold adapted forms showing a thick cocoon (*ca.* 25 µm).

LANGER & HANCE (2000), under the same experimental conditions (10˚C; L9 :D15), observed that the supercooling point, a common comparative cold tolerance measure (*e.g.* SALT, 1961; LEE, 1991), of white mummies (-26.0 ± 0.3˚C) was on average 1.1˚C higher than the value they obtained for dark mummies (-27.1 ± 0.7˚C). This significant difference may partly be explained by our results as it corresponds also to a significant difference of water content (Wf) between white and dark mummies. It is commonly assumed (*e.g.* SALT, 1961; CANNON & BLOCK, 1988; RING & DANKS, 1994) that insects reduce their water content before winter, while cryoprotectant contents generally increase, enhancing cold-hardiness significantly. Water content reduction is often a prerequisite for survival during exposure to extremes of cold.

According to SALT (1961) "cold-hardiness and diapause are separate phenomena, the relation between the two, arising from their co-occurrent timing". ADEDOKUN & DENLINGER (1984) demonstrated also that cold-hardiness and diapause are both commonly associated with overwintering, but that the relationship between the two is often obscure. This conclusion is also shared by VERNON & VANNIER (2002). Generally, insects show a complex and often interactive range of reactions to the deteriorating environmental conditions that occur as winter approaches. In temperate regions, where mild winters are quite frequent, a part of the *A. rhopalosiphi* population undergoes quiescence. The other part overwinters under a diapause state (LANGER & HANCE, 2000). In our experiment, the environmental conditions of temperature and photoperiod corresponded to autumnal conditions in Belgium. These conditions seemed not favorable to the induction of diapause. However, it is possible that in response to the deteriorating environmental conditions, the mummies with a longer development time were in quiescence representing a first step to diapause. The overlapping of development time, water content and cocoon thickness between colour morphs that we observed probably indicates different capacities to survive winter.

We presume that the colour of the mummy reflects the colour of the cocoon but also the colour of the cuticle of the dead aphid. The colour of the mummy is not only related to the diapause status but also to the aphid morph and its physiological status. A complementary possibility is that colour differences will be related to atmospheric humidity at the time of mummy formation (NOWBAHARI & THIBOUT, 1990). In a new experiment, we are currently attempting to identify the possible links between development time (diapausing criterion) and cocoon thickness (cold resistance criterion). We are also trying to relate these two criteria to other physiological or morphological characteristics using multivariate analyses.

ACKNOWLEDGEMENTS

This study was supported by the Service de Recherche Agronomique, Ministère des Classes Moyennes et de l'Agriculture, the FNRS (Belgium) and the Centre National de la Recherche Scientifique, Direction des Relations Internationales (France). The authors thank Joseph Le Lannic (Centre de Microscopie Electronique à Balayage et Micro-Analyse, Université de Rennes 1). We also thank Christophe Pels for technical assistance, Guy Boivin, Johan Burtin and Renate Wesselingh for constructive advice. This publication is publication BRC 019 of the Research Center on Biodiversity, UCL.

REFERENCES

ADEDOKUN, T.A. & D.L. DENLINGER (1984). Cold-hardiness : a component of the diapause syndrome in pupae of the flesh flies, *Sarcophaga crassipalpis* and *S. bullata*. *Physiol. Entomol.* 9 : 361-364.

CAMPBELL, A., B.D. FRAZER, N. GILBERT, A.P. GUTIERREZ & M. MACKAUER (1974). Temperature requirements of some aphids and their parasites. *J. Appl. Ecol.,* 11 : 431-438

CANNON, R.J.C. & W. BLOCK (1988). Cold tolerance of Microarthropods. *Biol. Rev.,* 63 : 23-77.

CHAUVIN, G. & G. VANNIER (1997). Supercooling capacity of *Tineola bisselliella* (Hummel) (Lepidoptera : Tineidae) : Its implication for disinfestation. *J. Stored Prod. Res.,* 33 : 283-287.

KRESPI, L., C.A. DEDRYVER, J.M. RABASSE, & J.P. NÉNON (1994). A morphometric comparison of aphid mummies containing diapausing vs. non-diapausing larvae of *Aphidius rhopalosiphi* (Hymenoptera : Braconidae, Aphidiinae). *Bull. Entomol. Res.,* 84 : 45-50.

KRESPI, L., C.A. DEDRYVER, V. CREACH, J.M. RABASSE, A. LE RALEC & J.P. NÉNON (1997). Variability in the development of cereal aphid parasitoids and hyperparasitoids in oceanic regions as a response to climate and abundance of hosts. *Environ. Entomol.,* 26 : 545-551.

LANGER, A., & T. HANCE (2000). Overwintering strategies and cold hardiness of two aphid parasitoid species (Hymenoptera : Braconidae, Aphidiinae). *J. Insect Physiol.,* 46 : 671-676.

LEATHER, S.R., K.F.A. WALTERS & J.S. BALE (1993). *The ecology of insect overwintering*. Cambridge University Press, Cambridge.

LEE, R.E. Jr. (1991). Principles of insect low temperature tolerance. In : *Insects at Low Temperature.* LEE JR., R.E. & D.L. DENLINGER (Eds). Chapman and Hall, New York and London : 17-46.

NOWBAHARI, B. & E. THIBOUT (1990). The cocoon and humidity in the development of *Acrolepiopsis assectella* (Lep.) pupae : consequences in adults. *Physiol. Entomol.,* 15 : 363-368.

RIGAUX, M., P. VERNON & T. HANCE (2000). Relationship between acclimation of *Aphidius rhopalosiphi* (De Stefani-Peres) in autumn and its cold tolerance (Hymenoptera : Braconidae, Aphidiinae). *Mededelingen van de Faculteit Landbouwwetenschap*, University of Gent, 65/2a : 253-283.

RING, R.A. & H.V. DANKS (1994). Desiccation and cryoprotection : overlapping adaptations. *Cryo-Letters*, 15 : 181-190.

Ruggle, P. & N. Holst (1994). Life history parameters of parasitoids attacking cereal aphids. *Norwegian Journal of Agricultural Sciences*, 16 : 83-88.

Salt, R.W. (1961). Principles of insect cold-hardiness. *Annu. Rev. Entomol.,* 6 : 55-73.

SAS Institute Inc (1990). SAS/STAT Users Guide, release 6.12 edition. Cary, NC, SAS Institue Inc.

Schlinger, E.I. & J.C. Hall (1959). A synopsis of the biologies of three imported parasites of the spotted Alfalfa aphid. *J. Econ. Entomol.*, 52 : 154-157.

Schlinger, E.I. & J.C. Hall (1960). The biology, behavior and morphology of *Trioxys* (*Trioxys) utilis*, an internal parasite of the spotted Alfalfa, *Therioaphis maculata* (Hymenoptera : Braconidae, Aphidiinae). *Ann. Entomol. Soc. Amer.,* 54 : 34-45.

Sigsgaard, L. (2000). The temperature-dependent duration of development and parasitism of three cereal aphid parasitoids, *Aphidius ervi*, *A. rhopalosiphi* and *Praon volucre*. *Entomol. Exp. Appl.,* 95 : 173-184.

Stàry, P. (1970). Biology of aphid parasites (Hymenoptera : Aphidiidae) with respect to integrated control. *Series entomologicae*, vol. 6, Dr. W. Junk Publishers, The Hague.

Tagawa J. (1996). Function of the cocoon of the parasitoid wasp, *Cotesia glomerata* L. (Hymenoptera : Braconidae) : Protection against dessication. *Appl. Entomol. Zool*., 31 : 99-103.

Vernon, P. & G. Vannier (1996). Developmental patterns of supercooling capacity in a subantarctic wingless fly. *Experientia*, 52 : 155-158.

Vernon, P. & G. Vannier (2002). Evolution of freezing susceptibility and freezing tolerance in terrestrial arthropods. *C. R. Biologies* 325 : 1185–1190.

Wiackowski, S.K. (1962). Studies on the biology and ecology of *Aphidius smithi* sharma & subba rao (Hymenoptera, Braconidae), a parasite of the pea aphid, *Acyrthosiphon pisum* (harr.) (Homoptera, Aphididae). *Bull. Entomol. de Pologne,* 21 : 253-310.

Worland, M.R., G. Grubor-Lajsic & P.O. Montiel (1998). Partial desiccation induced by sub-zero temperatures as a component of the survival strategy of the Arctic collembolan *Onychiurus arcticus* (Tulberg). *J. Insect Physiol.,* 44 : 211-219.

Belg. J. Zool., 134 (supplement 1) : 15-21 March 2004

Sex determination mechanism in the hymenopteran parasitoid *Aphidius rhopalosiphi* De Stefani-Peres (Braconidae : Aphidiinae)

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ABSTRACT. Three main sex-determining mechanisms have been proposed for Hymenoptera : genetic balance, single locus (sl-CSD) and multilocus (ml-CSD) complementary sex determination. In the last two cases, sex is not determined by the number of chromosome sets but by heterozygosity at one or several loci. Individuals are male when hemizygous (haploid) or homozygous (diploid) at all sex-determining loci. Usually, this results in haploids developing as males and diploids as females, although diploid males can also appear, particularly under conditions of inbreeding. *Aphidius rhopalosiphi* (Aphidiineae : Hymenoptera Braconidae) is a cereal aphid parasitoid that can potentially be used as a biological control agent. Phylogenetic studies suggested that, within parasitoid wasps, the sl-CSD is present in both the Ichneumonoidea superfamily and the Braconidae family. Here, we directly test the sl-CSD model in *A. rhopalosiphi* by inducing diploid male production by brother-sister mating in laboratory-selected isofemale lines. Ploidy levels were analyzed with two complementary methods : DNA flow cytometry and DNA microsatellite markers. We observed a significantly male-biased sex ratio after sib mating, but no diploid males were detected by DNA analysis. The difference between the observed and expected sex ratio suggests that a sl-CSD model with two alleles may be applicable, which would imply that most diploid males are unviable in *A. rhopalosiphi*. Consequences of diploid male production are discussed in terms of the evolutionary biology of Hymenoptera and aphid biological control.

KEY WORDS : Hymenoptera, Aphidiinae, Haplodiploidy, Complementary sex determination, Diploid male, Microsatellite markers, Flow cytometry.

INTRODUCTION

During the past decades, the study of sex-determining mechanisms in sexually reproducing organisms has played a key role in our understanding of evolutionary biology. Testimony of this is HARDY's (2002) recent book "Sex-ratio : concepts and research methods", which presents an exhaustive review of the subject. In haplodiploids such as Hymenoptera, reproduction is based on arrhenotoky. Males develop parthenogenetically from unfertilized haploid eggs while females develop from fertilized diploid eggs. This mechanism gives a high degree of maternal control over offspring sex-ratio (GODFRAY, 1994). Haplodiploidy also has important consequences for the evolution of sex allocation, mating systems, population ecology and social evolution (CHARNOV, 1982; COOK & CROZIER, 1995; PEN & WEISSING, 2002; WEST et al., 2002).

Mechanistically, four genetic models have been proposed to explain the linkage between ploidy and sex in the Hymenoptera : complementary sex determination (CSD; WHITING, 1943), genic balance (CUNHA & KERR, 1957), nucleo-cytoplasmic balance (CROZIER, 1971) and genomic imprinting sex determination (POIRIÉ et al., 1992). Of these, the CSD model seems to be the most widely distributed one, being reported for about fifty species of Parasitica and Aculeata (STOUTHAMER et al, 1992; COOK, 1993a; COOK & CROZIER, 1995). Under the CSD model, sex is determined by multiple codominant alleles at a single locus (sl-CSD) or at multiple loci (ml-CSD). If individuals are heterozygous at any one of the loci, they develop as females, but if they are homozygous (diploid) or hemizygous (haploid) at all loci, they develop as males. The existence of CSD is detected with inbreeding experiments by mother-son and brother-sister matings. Diploid male production can represent a significant load since diploid males are usually sterile (COOK & CROZIER, 1995; HENSHAW et. al., 2002) and often non-viable (PETTERS & METTUS, 1980). Presence of diploid males is strongly correlated to inbreeding and constitutes a genetic load for the population as it results in a biased sex-ratio (females mated to diploid males, like unmated females, produce only male offspring, COOK, 1993a).

Aphidius rhopalosiphi De Stefani-Peres (Braconidae : Aphidiinae) is a cereal aphid parasitoid that has attracted considerable interest for biocontrol purposes (LEVIE et al.,

2001). In mass rearing experiments, required for biocontrol via mass-release, we have observed a significantly male-biased sex-ratio (Levie & Hance, unpublished data). This could indicate the production of diploid males, possibly as a result of the occurrence of inbreeding in the rearing experiments. However, as yet, no formal investigation has been conduced on the sex-determining mechanism in *A. rhopalosiphi.*

Phylogenetic studies (Cook, 1993a; Cook & Crozier, 1995) have suggested that the CSD model is present in the parasitic superfamily of the Ichneumonoidea and in the Braconidae family, to which the Aphidiinae belong, and that single-locus CSD (sl-CSD) is probably the ancestral model in the Aculeate-Ichneumonoid clade (Cook & Crozier, 1995). However, single-locus CSD has not been formally demonstrated as the sex-determining mechanism for any member of the Aphidiinae.

The aim of the present study was to test whether sex-determination in *A. rhopalosiphi* can be explained by the single-locus CSD model. In order to increase homozygosity at the sex-determining locus and induce the production of diploid males, brother-sister crosses were produced. Presence of diploid males was tested using three different approaches, sex-ratio analysis, microsatellite genotyping and flow cytometry DNA analysis.

MATERIAL AND METHODS

Biological material

A. rhopalosiphi were collected in Belgium from parasitized *Sitobion avenae* Fabricius aphid larvae in September 2000. Laboratory cultures were established on aphids reared on *Triticum aestivum* L wheat. All rearing cultures were maintained at 20˚C, 60% relative humidity and 16-h light : 8-h dark cycles. Wheat was removed once per week and new aphid larvae (reared independently under the same laboratory conditions) were presented to adult parasitoids after each adult emergence.

Establishment of brother-sister crosses

Five isofemale lines were established in September 2002 by crossings between newly emerged virgin females with their brothers over five consecutive generations. For each line and generation, between three to five sibling matings were realized. As control, we also performed twenty random matings using freshly emerged adults from mummies isolated from mass rearing stocks. Matings were performed by placing each male and female pair in a capsule together with 100 stage 2 or 3 aphid larvae on wheat. To prevent superparasitism, adult parasitoids were removed after one to two days. Mummies appeared after 12 days of development and were isolated until emergence. Sex of newly emerged adults was determined as parent fecundity. Adults were fed for two days with a honey solution (5%). All parasitoids (parents and offspring) were stored at -80˚C for future DNA analysis.

Sex-ratio analysis

Because it is difficult to determine the sex-ratio at the egg stage, only the secondary sex-ratio could be recorded. Sex-ratio was estimated as number of males divided by total number of wasps. All-male progenies were excluded from the analysis to prevent confusion of all-male progenies of virgin females with diploid males (Beukeboom et al., 2000). As diploid males can be unviable, brood size and percentage of emerged mummies were compared between control and inbred crosses. Brood size was estimated by absolute number of mummies produced since we always presented 100 aphid larvae to wasps and analyzed results using a non parametric Mann-Whitney test. Sex-ratio and percentage of emerged mummies were analyzed using a general linear model (Wilson & Hardy, 2002). Data were presented in the form of binary responses (0 or 1) and a logistic regression was applied. Statistical analyses were performed using Minitab (version 12.2).

Expected sex-ratios under two different scenarios of sl-CSD (two and three alleles at the sex-determining locus) were calculated from the observed sex-ratio in the control crosses and compared to the observed sex-ratio in the first generation of inbred crosses (for a description of this method, see Beukeboom et al., 2000). Viability parameters of diploid males were also incorporated into the expected sex-ratio calculation (see Table 2). For example, the observed sex-ratio in the control crosses was 0.35 (n=210) and we obtained, in the first inbred generation, a total of 214 wasps (158 males and 56 females). If we considered sl-CSD model with two alleles at the sex-determining locus, 50% of the fertilized eggs are expected to become diploid males (i.e. 0.325) and expected sex-ratio (noted as SR, males proportion) is calculated as :

Case 1 SR1=(0.35 + 0.325) / (0.35 + 0.325 + 0.325); SR1=0.67 if diploid males are viable and

Case 2 SR2=0.35 / (0.35 + .0325); SR2=0.52 if diploid males are unviable.

In the first inbred generation, the number of observed wasps was 214 and expected male and female numbers (noted as Mnb and Fnb) are :

Case 1 Mnb1=214 x 0.67 and Fnb1=214 – Mnb1 if diploid males are viable

Case 2 Mnb2=214 x 0.52 and Fnb2=214 – Mnb2 if diploid males are unviable

Deviations from the observed and expected male and female numbers of wasps were tested using a chi-squared test with Yates'correction.

The same approach was realized with sl-CSD model with three alleles at the sex-determining locus but, in this case, 25% of the fertilized eggs are expected to become diploid males in the first inbred generation.

Discrimination between haploid and diploid males

Ploidy of male parasitoids was analysed by means of two complementary DNA analysis techniques : microsatellite genotyping of thorax and abdomen tissue and flow cytometry of the head tissue.

Microsatellite DNA genotyping

Diploid males can be identified by genotyping at several loci : if at least one locus is found to be heterozygous they are diploid rather than haploid. Because of their variability, we chose to use microsatellites as genetic markers. Six microsatellite DNA loci (polymorphic codomi-

nant markers) previously developed for *Aphidius ervi* and related species (AF336990, AF336991, AF336992, AF33693, AF336994, AF336998; HUFBAUER et al., 2001) were screened. The applicability on Belgian populations of *Aphidius rhopalosiphi* was previously verified by cloning and sequencing microsatellite loci.

DNA was extracted from thorax and abdomen using a CTAB extraction buffer, chloroform-isoamyl alcohol extraction and isopropanol precipitation. Microsatellite loci were amplified using the polymerase chain reaction. Amplification was performed in a 15 microlitre reaction mixture consisting of 2µl of genomic DNA, 1x PCR Buffer (10x), 2µM $MgCl_2$, 200µM dNTP, 0.5µM of each primer and 0.6 units AmpliTaq polymerase Gold (Applied Biosystems). All PCR reactions were performed using an Applied Biosystem : GeneAmp PCR system 9700, with a thermocycling profile consisting of a 10-min denaturation at 95˚C, 32 cycles of 94˚C for 50 s, 52˚C for 1 min and 72˚C for 1 minute 30s, followed by a final extension at 72˚C for 10 minutes. One primer for the locus was 5'-end-labelled with ABI PRISM® primer (Applied Biosystems). Allele sizes were determined by electrophoresis on polyacrylamide sequencing gels (ABI™ 377 DNA Sequencers), using a 400 HD ROX as a size standard. Gels were analyzed with GeneScan® software.

The efficiency of detecting diploid males among offspring could be determined by genotyping the parents. Initial genotyping of parents of the inbred crosses revealed that, of the six loci screened, only locus AF336990 was polymorphic. Furthermore, the identification of homozygous (hemizygous) and heterozygous genotypes was possible for only one line (line A) since a diploid female (mother, alleles 218-224bp.) presented one allele different to that of her mate (father, allele 218bp.) (see Fig. 2). From their genealogy, it appeared that males presenting a band at 224bp. must have a haploid genotype as this allele was only present in their parent female, but the haplodiploid states of males with a 218bp. band can not be defined as they could have either received this allele from their mother only and be hemizygous (haploid, genotype 218) or they were laid as homozygous diploid individuals receiving the 218 allele from both parents (diploid genotype 218/218). Thus, in the following brother-sister crosses, microsatellite markers allowed us to detect only 50% of all expected diploid males.

Flow cytometry DNA analysis

Flow cytometry was applied after nuclear preparation using a Becton Dickinson Facscan cytometer. We followed the protocol of VINDELOV et al. (1983) for nuclear preparations based on a trypsin solution followed by a trypsin inhibitor-ribonuclease solution and finally by a propidium-spermine tetrahydrochloride solution.

RESULTS

Secondary sex-ratio analyses

Results of first generation inbred (brother-sister crosses) and control crosses are presented in Table 1. The binary logistic regression analyses showed that a significant difference occurred between the mean secondary sex-ratio of control crosses and of the first inbred generation ($z=7.42$; $p < 0.001$). Thus, secondary sex-ratios in the first inbred generation (mean=0.38; SD=0.24) were more male biased than in the control crosses (mean=0.68; SD=0.22). No significant differences were found in the brood sizes (Mann-Whitney test, $W=135$, $p=0.87$) or percentage of emerged parasitoids (binary logistic regression : $p > 0.05$) between control and inbred crosses. No significant differences were observed in sex-ratio of the successive generations of brother-sister crosses (binary logistic regression : $p > 0.05$, Fig. 1). Moreover, we observed extinction of the isofemale line A in the fourth inbred generation.

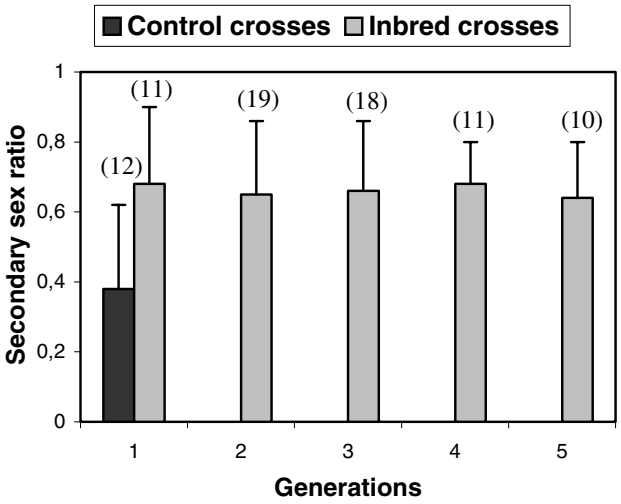

Fig. 1. – Secondary sex-ratio following inbreed crosses (sib mating) through five generations (mean ± SD). Comparison with control crosses. Numbers of crosses are indicated in parentheses.

TABLE 1

Description of the observed results (mean ± SD) in the control crosses and in the inbred crosses of the first generation: brood size (number of mummies), percentage of emerged mummies, mean number of females/pair, mean number of males/pair and secondary sex-ratio (males proportion).

	Control crosses (n=12)	Inbred crosses (n=11)
Brood size	18.00 ± 7.78	21.18 ± 16.38
Percentage of emerged mummies	92.69 ± 8.33	90.54 ± 10.86
Number of females	10.83 ± 7.12	5.09 ± 4.70
Number of males	5.92 ± 3.55	14.36 ± 15.27
Secondary sex ratio	0.38 ± 0.24	0.68 ± 0.22

Expected sex-ratio under sl-CSD assumptions

Expected secondary male sex-ratios, calculated from sex ratios observed in control crosses for different sl-CSD scenarios, are presented in Table 2. There was no significant difference between observed and expected sex-ratio under the assumption of sl-CSD based on two alleles at one single sex-determining locus with viable diploid males. In contrast, the observed sex-ratio was significantly more male-biased than expected if two alleles with non-viable diploid male and three sex alleles were postulated.

TABLE 2

Comparison between observed and expected numbers of males and females and sex ratio (males proportion) in control crosses and in first inbred generation with two and three alleles at the sex-determining locus. Viability of the diploid males were also integrated in the analysis. Significance of χ^2test were indicated as: NS, no significant difference and S, significant difference.

	Number of males and females	Sex-ratio (males proportion)	χ^2test (95%)
Control crosses	71 / 130	0.35 (n = 210)	
Observed results in the inbred crosses	158 / 56	0.74 (n = 214)	
sl-CSD: two alleles at the sex-determining locus			
Expected results with viable diploid male	144.45 / 69.55	0.67	NS
Expected results with non-viable diploid male	110.85 / 103.148	0.52	S
sl-CSD: three alleles at the sex-determining locus			
Expected results with viable diploid male	109.14 / 104.86	0.51	S
Expected results with non-viable diploid male	89.88 / 124.12	0.42	S

Search for diploid male production

Genealogical analysis with Microsatellite DNA markers

As explained previously, only locus AF336990 was polymorphic and the identification of homozygous (hemizygous) and heterozygous genotypes was possible for only one line (line A). Genotypes of parents, of the successive generations and of their male progeny are summarized in Fig. 2. Normally, those diploid males should be sterile, and if females mate with them, they

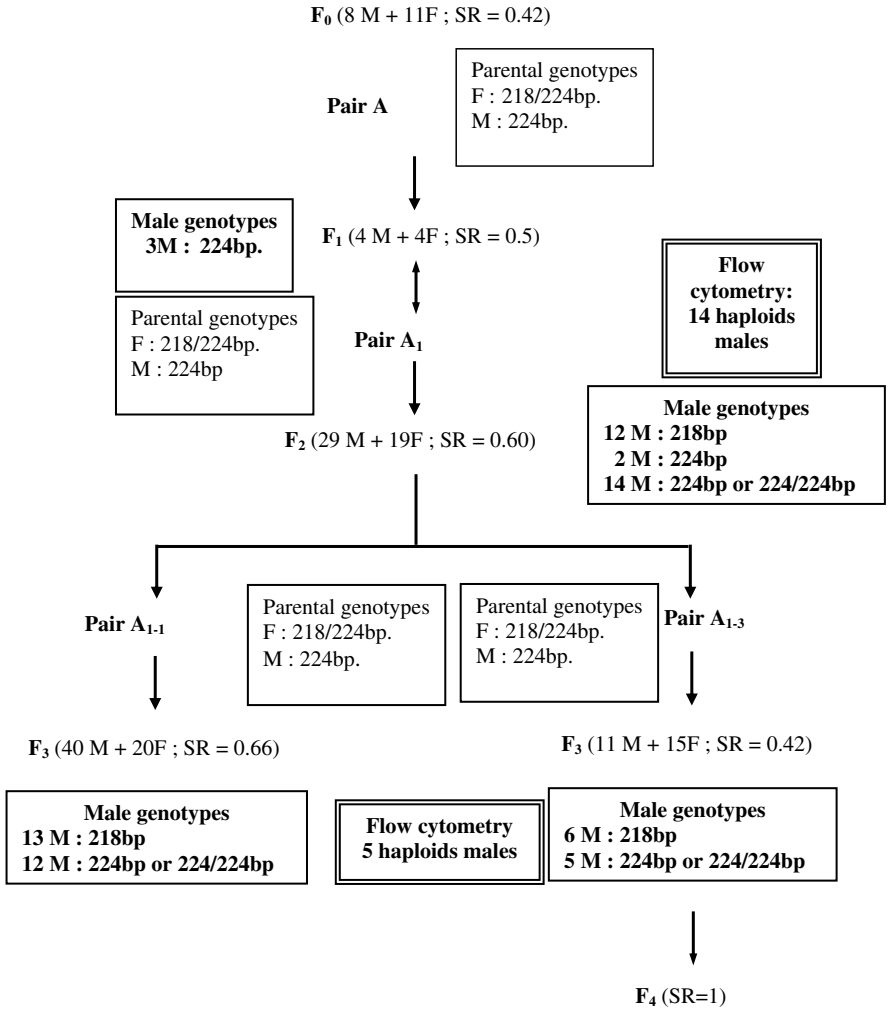

Fig. 2. – Genealogy of the isofemale lines using microsatellite DNA analysis. Secondary sex ratio (noted SR) and observed number of males are reported. Single line boxes represent parents genotypes whereas double line boxes represent genotypes of the male offspring. Results of flow cytometry analysis are also indicated.

should be able to produce haploid males only. In the first generation, three of the males had a haploid genotype as their progeny contained females (sex-ratio < 1). By the same reasoning, the second generation gave, of the 29 males genotyped : (i) 14 haploid males (12 with genotype 218 and two with genotype 224 (offspring tested) and (ii) 14 males that could either be haploid (genotype 224) or diploid (genotype 224/224). For the third generation, of the 11 males obtained, six were haploid and five could have been haploids or diploids.

Flow cytometry analysis

Flow cytometry was used to test the ploidy of males coming from generations 2 and 3 (generation 2 : 14 males; generation 3 : 5 males) that could not be resolved using microsatellites genotyping (for details, see Fig. 2). Before males were analyzed, several diploid females were tested as a control, so that the relative fluorescence index could be standardized (Fig. 3). None of the males tested by flow-cytometry, however, were diploid; all were normal haploid males. Hypotheses than can explain this observation are discussed in the following section.

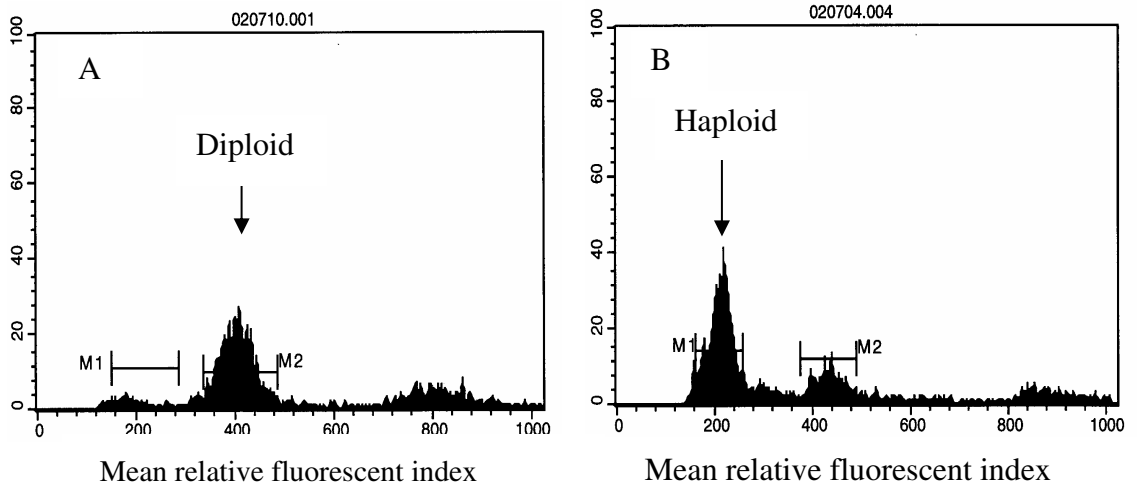

Fig. 3. – Profile of diploid female (A) and haploid male (B) using flow cytometry DNA analysis. M1 and M2 correspond to mean relative fluorescence peak of haploid and diploid cells respectively

DISCUSSION

This study presents the first investigation of the sex determination mechanisms in the braconid subfamily Aphidiinae. Experiments with laboratory cultures of *A. rhopalosiphi* showed that inbreeding results in a more male-biased secondary sex-ratio than that observed either in control crosses or under natural conditions (0.43 ± 0.03, Levie, unpubl.). Statistical analysis comparing observations with the expected sex-ratio under a sl-CSD assumption supported the simplest model based on two alleles at a single sex-determining locus with viable diploid males. Nevertheless, DNA microsatellite genotyping and flow cytometry analysis yielded useful complementary information on the ploidy of males since no diploid males were detected in the subsequent inbreeding generations of the analysed isofemale line. Even if these analyses do not allow us to draw clear conclusions on the sex determination mechanisms, our data suggest that sl-CSD with unviable diploid males is the likely sex determination mechanism in *A. rhopalosiphi*.

We propose here two nonexclusive explanations for the disparities between the results of the statistical analysis and the DNA analysis. First, under laboratory conditions, sex-ratio may be influenced by the existence of an inbreeding depression phenomenon. This hypothesis might be corroborated by the high male-biased secondary sex-ratio and the extinction of the isofemale line A in the fourth generation (only all-male progenies). Perhaps inbreeding depression might result in differential mortality of the sexes (high female mortality rate) explaining the absence of brood size differences between control and inbred crosses. However, if inbreeding depression occurs in haplodiploid systems, expression of lethal genes would be higher in haploid than diploid individuals and so would result in a higher mortality rate of haploid males. Inbreeding depression has already been reported within inbred *Diadegma chrysostictos* and *Trichogramma pretiosum* wasps (Antolin, 1999; Butcher et al., 2000).

A second explanation may be the absence of CSD sex-determining model in *A. rhopalosiphi* although low or non-viability of diploid males due to egg and larval mortality would also explain this pattern. Low viability of diploid males associated with CSD has been reported for Hymenoptera *Bracon hebetor* (Whiting, 1943; Petters & Mettus, 1980). In future, it may perhaps be possible to directly demonstrate differential mortality between haploid and diploid males, e.g. by comparing primary sex-ratio with secondary sex-ratio.

Moreover, Stouthamer et al. (1992) and Cook (1993a, 2002) underlined that wasp populations from laboratory cultures may have a lowered genetic diversity resulting from the fixation of several sex loci (homozygous). Populations may then contain only two sex alleles at one non-fixed sex locus. In this case, diploid male production would not increase in subsequent generations as the isofemale line cannot lose another sex allele or it will become extinct (Cook, 1993b) as observed in our laboratory culture (inbred generations 2 to 5, Fig. 1). Although our laboratory culture has been established for

only one year from specimens collected in nature, we cannot exclude the existence of some inbred crosses during that period or an initial reduced genetic diversity in the field samples. Thus we cannot reject definitively the existence of a ml-CSD model even if sex-ratio analyses correspond better to sl-CSD. Further investigations would be needed to exclude ml-CSD as the sex-determining mechanism in *A. rhopalosiphi*, since the number of generations and repetitions we used is probably insufficient to find diploid males.

Taking a phylogenetic overview, sl-CSD is likely to be the ancestral model in the Aculeate-Ichneumonoid clade (Cook, 1993a; Cook & Crozier, 1995). It has indeed been described in four species of the Braconidae family (three species from the Braconinae subfamily and one species from the Microsgasterinae subfamily, Speicher & Speicher, 1940; Whiting, 1943; Clark & Rubin, 1961; Steiner & Teig, 1989). However, phylogenetic patterns are still uncertain (see Wharton et al., 1992) especially since Beukeboom et al. (2000) first reported the existence of non-sl-CSD in a species belonging to the Alysiinae subfamily (Braconidae). Moreover, the Aphidiinae in concert with the Mesostoinae subfamilies form a subgroup in the cyclostomes Braconidae isolated from other subfamilies (Belshaw et al., 1998). We, therefore, plan further investigations on this subfamily, particularly on *A. rhopalosiphi* and the related *Aphidius ervi* species. These data should contribute to elucidation of the evolution of the CSD in the Braconidae family. DNA flow analysis on males collected from natural populations could bring interesting information on both the complementary sex determination model but also on mating strategy. Production of diploid males, which are sterile, in natural populations will generate a costly genetic load in haplodiploid species, and reduce the reproductive success of parents (Cook & Crozier, 1995; Zayed & Packer, 2001).

Industrial mass rearing is pivotal if cereal aphid parasitoids are ever to be used as biological control agents. However, knowledge of the sex determination mechanisms and sex-ratio biases could greatly reduce the costs of parasitoid production and increase the efficiency of mass-release programs.

ACKNOWLEDGEMENTS

The authors thank A. Ferreira Lima, MC. Flamand, C. Noel and B. Purnelle for their helpful assistance and comments on molecular techniques and analyses. This study was supported by a FSR grant from the Catholic University of Louvain and is publication BRC 021 of the Biodiversity Research Center, UCL. We greatly thank anonymous referees for constructive comments on an earlier version of this paper.

REFERENCES

Antolin, M.F. (1999). A genetic perspective on mating systems and sex ratios of parasitoid wasps. *Res. Popul. Ecol*., 41 : 29-37.

Belshaw, R., E. Herniou, C. Gimeno, M.G. Fitton & D.L.J. Quicke (1998). Molecular phylogeny of the Ichneumonoidea (Hymenoptera) based on D2 expansion region of 28S rDNA. *Syst. Entomol.*, 23 : 109-123.

Beukeboom, L.W., J. Ellers & J.M. Van Alphen (2000). Absence of single-locus complementary sex determination in the braconid wasps *Asobara tabida* and *Alysia manducator*. *Heredity*, 84 : 29-36.

Butcher, R.D.J., W.G.F. Whitfield & S.F. Hubbard (2000). Single locus complementary sex determination in diadegma chrysostictos (Gmelin) (Hymenoptera : Ichneumonidae). *J. Hered*., 91 : 104-111.

Charnov, E.L. (1982). *The theory of sex allocation*. Princeton, NJ : Princeton University Press.

Clark, A.M. & M.A. Rubin (1961). The modification by X-radiation of the life span of haploids and diploids of wasp, *Habrobracon* sp. *Radiation Res*., 15 : 224-253.

Cook, J.M. (1993a). Sex determination in the Hymenoptera : A review of models and evidence. *Heredity*, 71 : 421-435.

Cook, J.M. (1993b). Experimental tests of sex determination in *Goniozus nephantidis* (Hymenoptera : Bethylidae). *Heredity*, 71 : 130-137.

Cook, J.M. (2002). Sex determination in invertebrates. In : *Sex ratio : concepts and research methods*. Hardy, I.C.W. (Ed.), Cambridge University Press, Cambridge : 26-48.

Cook, J.M. & R.H. Crozier (1995). Sex determination and population biology in the Hymenoptera. *Trends Ecol. Evol*., 10 : 281-286.

Crozier, R.H. (1971). Heterozygosity and sex determination in haplodiploidy. *Am. Nat*., 105 : 399-412.

Cunha, A.B. & W.E. Kerr (1957). A genetical theory to explain sex-determination by arrhenotokous pathenogenesis. *Forma et Functio.*, 1 : 33-36.

Godfray, H.C.J. (1994). *Parasitoids. Behavioural and Evolutionary Ecology.* Princeton University Press, Princeton.

Hardy, I.C.W. (2002). *Sex Ratios, concepts and research methods*. Cambridge University Press, Cambridge (424 pp).

Henshaw, M.T., D.C. Queller & J.E. Strassmann (2002). Control of male production in the swarm-founding wasp, *Polybioides tabidus*. *J. Evol. Biol*., 15 : 262-268.

Hufbauer, R.A., S.M. Boddanowicz, L. Perez & R.G. Harrison (2001). Isolation and characterization of microsatellites in *Aphidius ervi* (Hymenoptera : Braconidae) and their applicability to related species. *Mol. Ecol*., 1 : 197-199.

Levie, A., P. Dogot & T. Hance (2001). Release of *Aphidius rhopalosiphi* (Hymenoptera : Aphidiinae) for cereal aphid control : field cage experiments. *Eur. J. Entomol*., 97 : 527-531.

Pen, I. & F.J. Weissing (2002). Optimal sex allocation : steps towards a mechanistic theory. In : *Sex ratio : concepts and research methods*. Hardy, I.C.W. (Ed.), Cambridge University Press, Cambridge : 26-48.

Petters, R.M. & R.V. Mettus (1980). Decreased diploid viability in the parasitoid wasp, *Bracon hebetor*. *J. Hered*., 71 : 353-356.

Poirié, M., G. Périquet & L. Beukeboom (1992). The hymenopteran way of determining sex. *Semin. Dev. Biol*., 3 : 357-361.

Speicher, B.R. & K.G. Speicher (1940). The occurrence of diploid males in *Habrobracon brevicornis*. *Am. Nat*., 74 : 379-382.

Steiner, W.W.M. & D.A. Teig (1989). *Microplitis croceipes* : genetic characterisation and developing insecticide resistant biotypes. *Soutwest Entomol*., 12 : 71-87.

Stouthamer, R., R.F. Luck & J.H. Werren (1992). Genetics of sex determination and the improvement of biological control using parasitoids. *Environ. Entomol*., 21 : 427-435.

Vindelov, L.L., I.J. Chritensen & N.I. Nissen (1983). A detergent-trypsin method for the preparation of nuclei for flow cytometric DNA analysis. *Cytometry*, 3 : 323-327.

West, S.A., S.E. Reece & B.C. Sheldon (2002). Sex ratio. *Heredity*, 88 : 117-124.

Wharton, RA., S.R. Shaw, M.J. Sharkey, D.B. Wahl, J.B. Wooley, J.B. Whitfield, P.M. Marsh & W. Johnson (1992). Phylogeny of the subfamiliy Braconidae (Hymenoptera : Ichneumonoidea) : a reassessment. *Cladistics*, 8 : 199-235.

Wilson, K. & C.W. Hardy (2002). Statistical analysis of sex ratio data. In : *Sex ratio : concepts and research methods*. Hardy, I.C.W. (Ed.). Cambridge University Press, Cambridge : 26-48.

Whiting, P.W. (1943). Multiple alleles in complementary sex determination of *Habrobacon*. *Genetics*, 28 : 365-382.

Zayed, A. & L. Packer (2001). High levels of diploid male production in a primitively eusocial bee (Hymenoptera : Halictidae). *Heredity*, 87 : 631-636.

Belg. J. Zool., 134 (supplement 1) : 23-28 March 2004

Cannibalism and prey sharing among juveniles of the spider *Oedothorax gibbosus* (Blackwall, 1841) (Erigoninae, Linyphiidae, Araneae)

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ABSTRACT. In a terrarium experiment, juvenile cannibalism in the dwarf spider *Oedothorax gibbosus* (Blackwall, 1841) was very high – up to 99 %. More first juveniles were cannibalised at higher initial densities, and there was no effect on this cannibalism from offering different prey species. Although in their natural environment spiderlings may more readily evade one another, this terrarium experiment demonstrates the drastic effect of juvenile cannibalism. Larger first juveniles are apparently more prevalent among the cannibals, and juvenile cannibalism among similar sized spiderlings is less frequent. Prey sharing observed between similar sized first juveniles is not a social behaviour, but is rather a forced prey sharing resulting from competition among equally strong juveniles that are unable to defend their prey items from one another. Larger first juveniles do not tolerate smaller ones sharing their prey.

KEY WORDS : Araneae, Erigoninae, juvenile cannibalism, food sharing, competition.

INTRODUCTION

According to Polis (1981), cannibalism is killing and consuming an individual or a part of it that belongs to the same species. Elgar & Crespi (1992) mention different forms of cannibalism, such as cannibalism in a context of competitive interaction, during copulation and courtship behaviour, killing of offspring by one or both parents (infanticide), eating of older individuals (gerontophagy), consumption of eggs (oophagy) and cannibalism between juveniles (juvenile or sibling cannibalism). Cannibalism, including juvenile cannibalism, occurs in many taxa (Elgar & Crespi, 1992). Juvenile cannibalism has been reported in Arachnida (Elgar & Crespi, 1992), Odonata (Crowly et al., 1987), Thysanopthera (Crespi, 1992), Heteroptera (Banks, 1968; Nummelin, 1989; Orr et al., 1990), Trichoptera (Mecom, 1972), Lepidoptera (Cottrell, 1984; Jasienski & Jasienska, 1988), Neuroptera (Duelli, 1981), Coleoptera (Crespi, 1992), Hymenoptera (Crespi, 1992), Osteichthyes (Sargent, 1992), Amphibia (Crump, 1992) and Reptilia (Polis & Myers, 1985).

Juvenile cannibalism is known for different spider species. Wagner & Wise (1996, 1997) described juvenile cannibalism in the wolf spiders *Schizocosa ocreata* (Hentz, 1844) and *Schizocosa stridulans* Stratton, 1984. Another record of juvenile cannibalism in wolf spiders is by Samu et al. (1999) in the species *Pardosa agrestis* (Westring, 1861). According to the latter, different factors possibly influence the extent of juvenile cannibalism, such as differences in body weight, hunger, life phase and age. They proved that juvenile cannibalism was strongly positively correlated with both weight ratio between involved cannibals and hunger, but absolute size or age of an individual could not predict the occurrence of a juvenile cannibalistic event (Samu *et al*. 1999). Juvenile cannibalism also occurs in the salticid *Menemerus bracteatus* (L. Koch, 1879) (Rienks, 2000). This spider species builds nests with different clutches and therefore the juveniles have the opportunity to cannibalise on 'sibling' eggs, prelarvae and larvae. Matriphagy, the cannibalism of the female by its offspring, probably also occurs in this spider species (Rienks, 2000). Cannibalism might be an important phenomenon in the regulation of populations (Samu *et al*., 1999). Also Wagner & Wise (1996,1997) argue that juvenile cannibalism could be an important mortality- and density-regulating factor in the studied wolf spider *Schizocosa*.

While taking care of *Oedothorax gibbosus* (Blackwall, 1841) spider cultures, we observed two first instar juveniles feeding on the same prey item, a springtail. This could represent helping behaviour in dwarf spiders (voluntary prey sharing hypothesis), or perhaps these first instar juveniles are unable to defend their prey against rival spiders (forced prey sharing hypothesis). Neither Polis (1981) nor Elgar & Crespi (1992) reported helping behaviour and juvenile cannibalism in the same species. Helping behaviour is typical for social spiders, i.e. spiders with co-operation between mutually tolerant individuals (Downes, 1995). A number of spider species live in groups, but few of them satisfy the criterion of social behaviour : co-operation in prey capture and brood care.

Krafft (1971) described prey capture in the social spider *Agelena consociata* Denis, 1965. A moving prey caught in the web draws the attention of all spiders that live in this web. One or more spiders attack the prey according to the prey size and the prey is carried into the common refuge where the actual feeding starts.

Also the social spider *Mallos gregalis* (Simon, 1909) exhibits similar feeding behaviour (Jackson, 1979). The aforementioned social spiders do not exhibit any kind of aggression during feeding. This lack of aggression is, however, not typical for all social spiders (Foelix, 1996). A social pholcid spider found in Malaysia exhibits some communal behaviour with web repair and defence, but individuals are competitively aggressive rather than cooperative in prey capture (Tomoij & Maryati, 2001). The social spider *Delena cancerides* Walckenaer, 1837 (Sparassidae) exhibits not only intra-nest tolerance and communal feeding behaviour, but also extreme aggression towards members of foreign colonies (Rowell & Aviles, 1995).

In this publication we describe the extent of juvenile cannibalism, and the effects of initial spider density and of diet on juvenile cannibalism in the dwarf spider *Oedothorax gibbosus*. We also analyse the aforementioned observed prey sharing in this species in more detail; on the basis of experiments with first instar juveniles of different sizes we weigh up the voluntary and forced prey sharing hypotheses.

MATERIAL AND METHODS

Oedothorax gibbosus individuals were caught in the nature reserve Het Walenbos in Tielt- Winge (50˚ 55' NL, 4˚ 51' EL) (Belgium), 30 km north-east of Brussels, one of the most important river-associated woodlands of Flanders. *O. gibbosus* occurs in this nature reserve in an oligo- to mesotrophic alder carr (De Keer & Maelfait, 1989; Alderweireldt, 1992). The dwarf spiders were caught by hand on October 13 and 27, 2001 and on April 29, 2002. Spiders used in the first, second and third experiments were of the first generation coming from female spiders inseminated in the field or from the first laboratory generation. The spiders used in the fourth and fifth experiment were of the second laboratory generation. All spiders were kept in a climatic chamber, photoperiod L :D 16 :8 and temperature of 20˚C.

Experiment 1. To determine the extent of juvenile cannibalism, all juvenile spiders hatched from the same cocoon were left together in one small plastic vial (5 cm diameter and 2.5 cm height) with a thin basal layer of plaster, regularly moistened to maintain relative humidity near 100%. We fed the spiders two springtails, *Sinella curviseta*, per day per surviving individual. We monitored the occurrence of cannibalism between juveniles from 111 cocoons, and noted the number of spiders present until those that were not cannibalised reached adulthood.

Experiment 2. Here we studied the effect of the initial density of spiders and of the diet composition on the extent of juvenile cannibalism. The spiders coming from a single cocoon were divided as follows into separate small vials of the kind described above : one spider in one vial, two spiders in one vial, three, and so on per vial until all spiders of one cocoon were used. The maximal density was seven. To investigate if diet composition has an effect on the extent of juvenile cannibalism we used four different diets. A first group of spiders received two springtails, *Sinella curviseta*, per day per surviving spider. A second group received two *S. curviseta* per spider per day, and after the second moult each spider received three fruit flies each day. This is the so-called successive *S. curviseta*-fruit fly diet. A third group was fed with four springtails, *Isotoma viridis*, each day. A fourth group received a simultaneous *S. curviseta*-fruit fly diet; four *S. curviseta* and three fruit flies per remaining spider per day. Table 1 shows the number of vials monitored for the several density categories and diets. Again, we noted the number of surviving juveniles until the spiders that were not cannibalised reached adulthood.

TABLE 1

The number of studied cups for the studied spider density categories and diets (mono-diet *Sinella curviseta*, successive poly-diet *S. curviseta*-fruit flies, mono-diet *S. curviseta* and simultaneous poly-diet *S. curviseta*-fruit flies) in the second experiment (See Material and methods).

diet initial density	*S. curviseta*	successive *S. curviseta* - fruit flies	*I. viridis*	simultaneous *S. curviseta* - fruit flies
1	21	5	5	7
2	25	7	9	5
3	23	4	4	7
4	20	7	6	3
5	7	5	5	5
6	7	3	5	2
7	3	2	2	2

Experiment 3. In a third experiment we used large rectangular vials (terraria) to more closely imitate the natural environment. Based on the number of cannibalised spiders per terrarium after 20 days, we determined whether more juvenile spiders were cannibalised with higher initial densities. We put between 112 and 156 recently hatched spiders from a breeding stock into each of six terraria. Each terrarium contained spiders hatched the same day from different cocoons (max number of hatched *O. gibbosus* individuals per cocoon = 40). The terraria were large rectangular plastic vials (17.5 cm length; 12.5 cm width and 6.5 cm height) with a thin basal layer of plaster and tufts of moss. These terraria were moistened regularly to maintain a relative humidity near 100%. In each terrarium there was also a culture of *S. curviseta*; because the springtails propagated, there was always an over-abundance of food. Each few days we provided dry yeast as food for the springtails.

Experiment 4. To study the extent and variation of prey sharing we observed, during five hours, recently hatched juveniles from ten cocoons in the above-mentioned small vials. Each vial initially contained ten first instar spiderlings of comparable size from the same cocoon, 15 *S. curviseta* and five *I. viridis* for prey.

Experiment 5. To investigate if prey sharing is voluntary or forced we observed, during five hours, 15 small vials, each with two first instar juvenile spiders of comparable size, and 15 vials with two first instar juvenile spiders of different sizes. In each vial there were four *S. curviseta* per spider.

Experiment 6. Is there more juvenile cannibalism between juvenile spiders of different sizes than between spiders of comparable sizes? In each of 25 small vials (5 cm diameter and 2.5 cm height) we placed two first instar juveniles of comparable size, and in another 25 vials we placed two first instar juveniles of different size. Over five

days we monitored the number of spiders present each day. *S. curviseta* individuals were added each day as necessary to maintain four springtails per spider per vial.

Data on cannibalistic performance in the second experiment were used as poisson distributed response variables in a generalized mixed linear model with log link (glimmix procedure in SAS 8.1) and backwards elimination of non-significant variables. The diet treatment was included as fixed class factors, the initial number of spiderlings and the observation period (developmental period till adulthood) as continuous variables. For analysis of the other experiments we used linear regression, and to compare percentages, χ^2-test; these analyses were performed with Statistica.

RESULTS

Experiment 1 : Extent of juvenile cannibalism

Juvenile cannibalism occurred in 110 of the 111 groups of juvenile descendants from individual cocoons kept together in small vials (99%).

Experiment 2 : Effect of initial density of juveniles and diet on juvenile cannibalism

Table 1 shows the numbers observed for the different spider density and diet categories. The degree of juvenile cannibalism depended significantly on initial density, total observation period, and the interaction between the two (Fig. 1 & 2). No variation was explained by the diet (Fig. 1) or the interaction of diet with the continuous variables. The goodness-of-fit of the model was significant (χ^2_{202} = 125, *NS*) and standardised residuals approached normality (Shapiro Wilk's W = 0.97). Table 2 shows the results of the linear model. Fig. 2 shows the relative number of cannibalised spiders from the start until adulthood and clearly demonstrates that more spiders were cannibalised at higher densities.

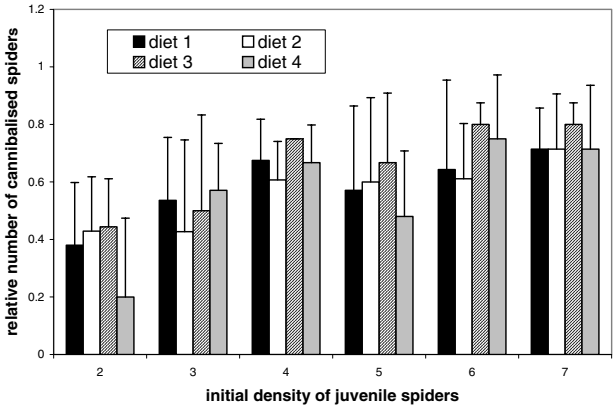

Fig. 1. – Histogram with means and standard deviations of number cannibalised spiders/total number of spiders between the four different diets and this for the different initial juvenile densities (1 = mono-diet *Sinella curviseta*, 2 = successive poly-diet *S. curviseta*-fruit flies, 3 = mono-diet *Isotoma viridis*, 4 = simultaneous poly-diet *S. curviseta*-fruit flies).

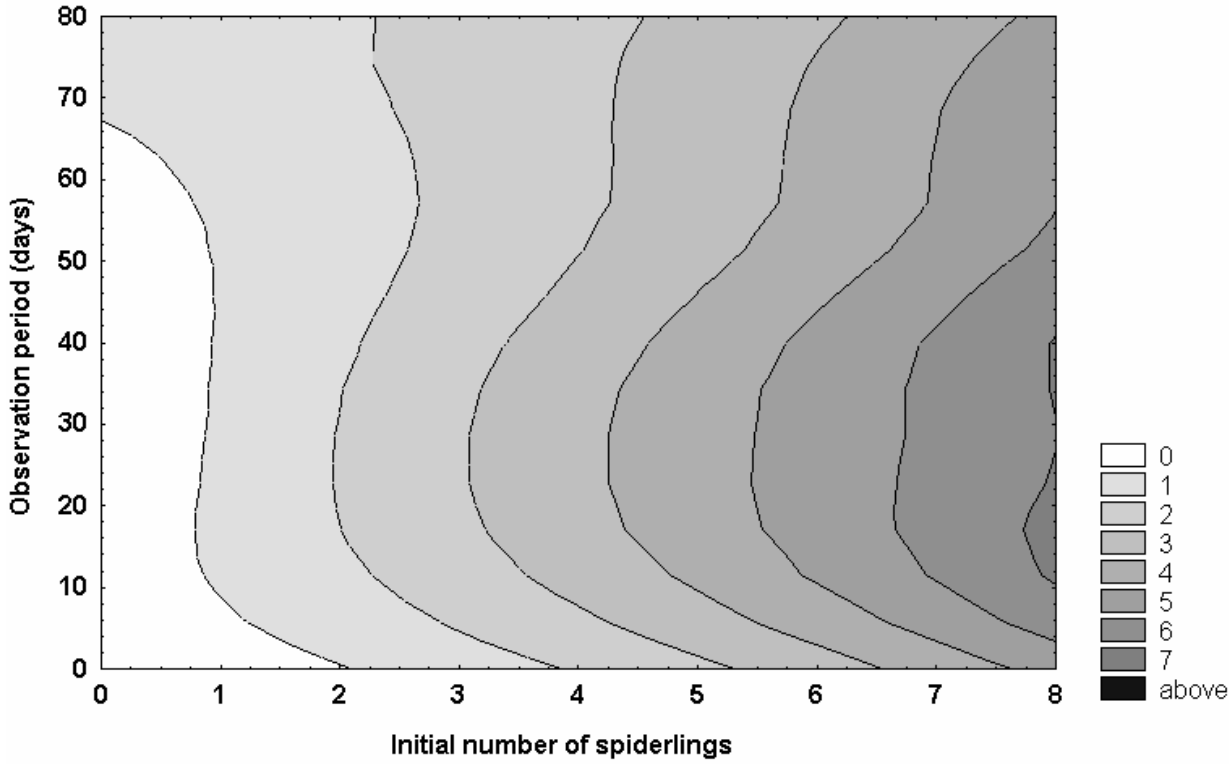

Fig. 2. – The number of cannibalised spiders of the second experiment (different grey values) is affected by the initial juvenile density, the number of observation days and the interaction between both.

TABLE 2

Statistical analysis of the effect of initial density of juveniles and diet on juvenile cannibalism (Experiment 2). Results of GLIMMIX model with stepwise backwards elimination of the non-significant contributions. Diet was treated as fixed factor; initial number of spiderlings and observation period were considered continuous variables.

Factor	num. df	den. df	F	P	R
Diet	3	202	1,12	0,343	
Initial density of spiderlings	1	202	164,37	**< 0,0001**	+0,866
Observation period (days)	1	202	14,24	**0,0002**	+0.242
Observation period x Initial density of spiderlings	1	202	19,47	**< 0,0001**	
Diet x Initial density of spiderlings	3	193	0,51	0,674	
Diet x Observation period	3	199	2,20	0,612	
Diet x Observation period x Initial density of spiderlings	3	196	0,53	0,661	

Experiment 3 : Terrarium experiments

We compared the mortality of *O. gibbosus* in six separate terraria after 20 days. Because there was an overabundance of springtails, we can consider that mortality was mostly due to juvenile cannibalism. After 20 days the number of spiders in each terrarium was drastically reduced, with mortality between 61% and 90%.

The initial number of spiders had a significant, positive effect on the extent of juvenile cannibalism (linear regression : $F_{1,4} = 12.93$; R = + 0.872; p = 0.023) (Fig. 3); this confirms the results found in the aforementioned juvenile cannibalism experiments.

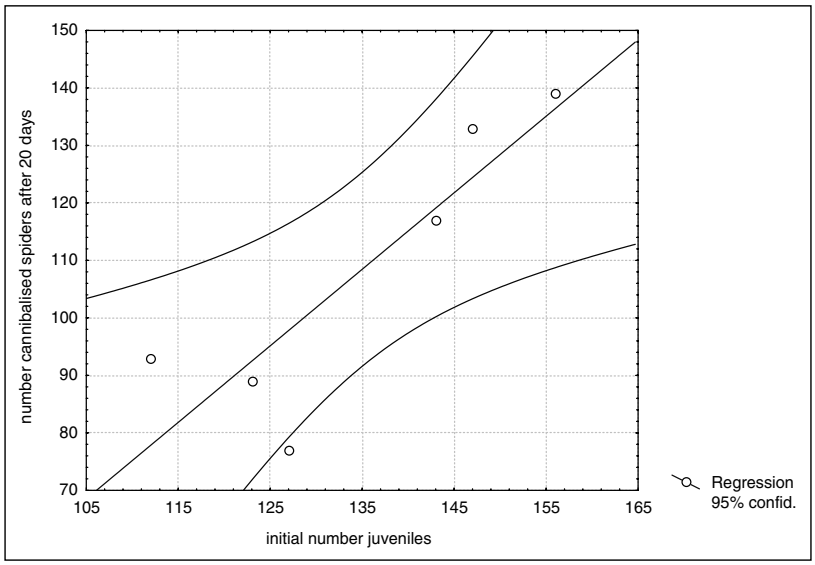

Fig. 3. – Bivariate correlation of the number of cannibalised juveniles after 20 days on the initial number of juveniles.

Experiment 4 : Variation in and extent of prey sharing

We observed ten vials each with ten first instar juvenile spiders of comparable size during five hours. Prey sharing between juveniles of *O. gibbosus* occurred five times. One juvenile caught the prey, namely a *S. curviseta*, and tolerated another juvenile eating the same springtail. In another case the springtail was shared among three juveniles. At first, the third juvenile was driven away by the other two, but a second attempt was successful and this resulted in prey sharing among three.

Once we observed prey sharing between an *O. gibbosus* juvenile and two springtails; a juvenile spider caught an *I. viridis* springtail and shared this prey with two *S. curviseta* springtails. This is exceptional; mostly the spiders drive the springtails away while eating.

There were also five observations of scavenging; different juvenile spiders fed successively on the same prey item. One spider always caught the springtail and started to eat it. After a while the spider left the half eaten springtail; soon a second spider began to eat this springtail. Scavenging cannot be considered as helping behaviour; in the next experiment we concentrated on real prey sharing.

Experiment 5 : Voluntary or forced prey sharing?

Observations on 15 small vials each with two first instar juveniles of comparable size and 15 vials each with two first instar juveniles of clearly different size showed that prey sharing occurred significantly more between spiderlings of comparable size (6/15) than between those of different size (0/15) ($\chi^2_1 = 5.14$; p = 0.023). Twice a small spiderling tried to eat from a prey caught by a larger

spiderling, but the latter did not tolerate this and drove the small one away.

Experiment 6 : Are cannibals mostly bigger than their victims?

To study this we compared the occurrence of juvenile cannibalism during five days between two first instar juvenile spiders of comparable size (10/25) and between two first instar spiderlings of different size (25/25); this difference was significant ($\chi^2_1 = 3.90$; $p = 0.048$).

DISCUSSION

The extent of juvenile cannibalism in the dwarf spider *Oedothorax gibbosus* is certainly very high; it occurred in 99 % of the cases in the first experiment. That juvenile cannibalism is very pronounced was also shown in the terrarium experiment (Exp. 3). After 20 days there was already a drastic decline of juvenile spiders; this mortality is most probably almost completely due to juvenile cannibalism. A slight overestimation of the extent of juvenile cannibalism is possible, because spiderlings in nature may be more able to evade one another. *O. gibbosus* spiders are, however, distributed in aggregations in alder carrs, such that the results given here are most probably a good indication for the extent of juvenile cannibalism in the field.

The second experiment showed a significant effect of initial juvenile density on the extent of juvenile cannibalism; more juveniles were cannibalised when initial density was higher. This result was confirmed by the terrarium experiment. Because we studied each case of the second experiment until adulthood of the cannibals, the number of observation days differed between cases, and the degree of juvenile cannibalistic behaviour also depended significantly on the number of observation days. Evidently, a longer juvenile development enhances chances for juvenile cannibalism. The different diets provided in the second experiment did not have a significant effect on the occurrence of juvenile cannibalism, possibly because they did not differ sufficiently in food quality.

According to the sixth experiment, it is mostly larger first juveniles that eat smaller ones, but juvenile cannibalism among similar sized juveniles also occurs. Size difference between first juveniles thus has important consequences for juvenile cannibalism, but also for the observed prey sharing in *O. gibbosus.* This is in agreement with Samu *et al.* (1999) : if cannibalism occurs among juvenile *Pardosa agrestis* spiders, the heavier spider is always the cannibal.

The fifth experiment shows that prey sharing occurs more among juveniles of comparable size; larger juveniles do not tolerate prey sharing with small ones and drive them away. This is an indication that the observed prey sharing is not voluntary; small juveniles are unable to defend their prey against rivals. This forced prey sharing is thus a consequence of the lack of a size difference between cannibalistic juveniles and is not an example of social behaviour. We observed this forced prey sharing in five of the ten cases in the fourth experiment and in six of the 15 cases with similar sized juveniles in the fifth experiment. This confirms again the importance of juvenile cannibalism in *O. gibbosus*.

Some observations in the fourth experiment confirm this "involuntary sharing" hypothesis. In one of the five cases of food sharing observed there was also prey sharing among three similar sized juveniles; the third juvenile spider was first driven away but a second attempt by this spider to eat part of the prey was successful. This indicates again the inability of small first juvenile spiders to defend their prey. The observation that one juvenile spider "shared" food with two springtails also confirms that food sharing is forced and not voluntary among cannibalistic juveniles in *O. gibbosus.*

ACKNOWLEDGEMENTS

We are indebted to Dr. Michael L. Draney of the Department of Natural and Applied Sciences of the University of Wisconsin-Green Bay for delivering several Collembola of the species *Sinella curviseta* to start a Collembola culture in our laboratory. We also thank Etienne Bijttebier for taking care of the *Sinella curviseta* culture. We also thank Liesbeth Maes, Domir De Bakker, Viki Vandomme, Frederik Hendrickx, Lut Van Nieuwenhuyse, Marjan Speelmans and Wannes Heirbaut for their help in catching the dwarf spiders in the field. The first author has a PhD grant of the Foundation for Scientific Research – Flanders.

REFERENCES

Alderweireldt, M. (1992). Determinatieproblematiek van de zustersoorten van het genus *Oedothorax* (Araneae, Linyphiidae). *Newsbr. Belg. Arachnol. Ver.*, 9 : 19-26.

Banks, C.J. (1968). Cannibalism and predation by aphids. *Nature*, 218 : 491.

Cottrell, C.B. (1984). Aphytophagy in butterflies : its relationship to myrmecophily. *Zool. J Linn. Soc.,* 79 : 1-57.

Crespi, B.J. (1992). Cannibalism and trophic eggs in subsocial and eusocial insects. In : *Cannibalism, ecology and evolution among diverse taxa*. Elgar, M.A. & B.J. Crespi (Eds). Oxford science publications, Oxford : 176-213.

Crowley, P., R. Nisbet, W. Gurney & J. Lawton (1987). Population regulation in animals with complex life histories : formulation and analysis of a damselfly model. *Adv. Ecol. Res.*, 17 : 1-59.

Crump, M.L. (1992). Cannibalism in amphibians. In : *Cannibalism, ecology and evolution among diverse taxa*. Elgar, M.A. & B.J. Crespi (Eds). Oxford science publications, Oxford : 256-277.

De Keer, R. & J-P. Maelfait (1989). Ecological backgrounds for the distribution patterns of *Oedothorax fuscus* (Blackwall) and *Oedothorax gibbosus* (Blackwall) (Erigoninae, Araneae). *Proceedings Symp. Inv. Belgium* : 189-192.

Downes, M.F. (1995). Australasian social spiders : what is meant by "social"? *Rec. West. Austral. Mus. Suppl.* : 25.

Duelli, P. (1981). Is larval cannibalism in lacewings (*Chrysopa carnea*) adaptive (Neuroptera : Chrysopidae)? *Res. Pop. Biol.,* 23 : 193-209.

Elgar, M.A. & B.J. Crespi (1992). *Cannibalism, ecology and evolution among diverse taxa*. Oxford science publications, Oxford (338 pp).

Foelix, R.F. (1996). *Biology of spiders*, 2nd Ed. Oxford University Press, Oxford.

Jackson, R.R. (1979). Comparative studies of *Dictyna* and *Mallos* (Araneae, Dictynidae) II. The relationship between courtship, mating, aggression and cannibalism in species

with differing types of social organization. *Rev. Arachnol.*, 2(3) : 103-132.

Jasienski, M. & G. Jasienska (1988). Parental correlates of the variability in body mass at hatching in *Arctia caja* larvae (Lepidoptera : Arctiidae). *Entomol. Genet.*, 13 : 87-93.

Krafft, B. (1971). La société d'*Agelena consociata* Denis, araignée sociale du Gabon. *Congr. Nat. Soc. Savantes, Toulouse :* 96.

Mecom, J.O. (1972). Feeding habits of Trichoptera in a mountain stream. *Oikos*, 23 : 401-407.

Nummelin, M. (1989). Cannibalism in waterstriders (Heteroptera : Gerridae) : is there kin recognition? *Oikos*, 56 : 87-90.

Orr, B., W. Murdoch & J. Bence (1990). Population regulation, convergence and cannibalism in *Notonecta* (Hemiptera). *Ecology*, 71 : 68-82.

Polis, G.A. 1981. The evolution and the dynamics of intraspecific predation. *Annu. Rev. Ecol. Syst.,* 12, 225-251.

Polis, G. & C.A. Myers (1985). A survey of intraspecific predation among reptiles and amphibians. *J. Herpetol.*, 19 : 99-107.

Rienks, J.H. (2000). Extended nest residence and cannibalism in a jumping spider (Araneae, Salticidae). *J. Arachnol.,* 28 : 123-127.

Rowell, D.M. & L. Avilez (1995). Sociality in a bark-dwelling huntsman spider from Australia, *Delena cancerides* (Walckenaer) (Araneae, Sparassidae). *Insect. Soc.,* 42 : 287-302.

Samu, F., S. Toft & B. Kiss (1999). Factors influencing cannibalism in the wolf spider *Pardosa agrestis* (Araneae, Lycosidae). *Behav. Ecol. Sociobiol.,* 45 : 349-354.

Sargent, R.C. (1992). Ecology and filial cannibalism in fish : theoretical perspectives. In : Elgar, M.A. & B.J. Crespi (Eds). *Cannibalism, ecology and evolution among diverse taxa*. Oxford science publications, Oxford : 38-63.

Tomoji, E. & M. Maryati (2001). A group-living spider (Araneae : Pholcidae) inhabiting the bases of large buttressed trees in the tropical rain forest of Sabah Malaysia. *Nature and Human Activities*, 6 : 41-48.

Wagner, J.D. & D.H. Wise (1996). Cannibalism regulates densities of young wolf spiders : evidence from field and laboratory experiments. *Ecology,* 77 : 639-652.

Wagner, J.D. & D.H. Wise (1997). Influence of prey availability and conspecifics on patch quality for a cannibalistic forager : laboratory experiments with the wolf spider *Schizocosa*. *Oecologia*, 109 : 474-482.

Belg. J. Zool., 134 (supplement 1) : 29-35 March 2004

Comparative study of courtship and copulation in five *Oedothorax* species

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ABSTRACT. *Oedothorax gibbosus* (Blackwall, 1841) is a dwarf spider restricted to oligo- and mesotrophic alder carrs. This dwarf spider is distinguished from other *Oedothorax* species by its male dimorphism; *gibbosus* males are characterised by a hunch and a hairy groove on their carapace, *tuberosus* males do not have these features. The hairy groove is important during gustatorial courtship behaviour; this is the uptake of secretions by the female from a male body part during courtship. Another remarkable difference between *O. gibbosus* and its sister species is that courtship and copulation duration is much longer in this species. The rare *O. gibbosus* spiders also seem to be less active than common spiders of *O. fuscus* (Blackwall, 1834), *O. retusus* (Westring, 1851) and *O. apicatus* (Blackwall, 1850). In that way common spiders raise their chances of survival in the open field. A more active lifestyle seems to be related to a shorter, heavier courtship and copulation. It is also possible that the longer courtship and copulation time, useful for male competition and sperm competition, results from the male dimorphism in this species. The web has an important function in the reproduction of *Oedothorax* spiders : it is necessary during gustatorial courtship, other courtship activities and copulation, and it is also probably important for the distribution of contact pheromones. The copulation of *Oedothorax* species differs from that of *Erigone* : instead of two continuous insertions, we observed, in this case, a series of very short insertions, which can be linked to one other to make up longer periods.

KEY WORDS : *Araneae, Erigoninae, Oedothorax, Erigone,* gustatorial courtship, courtship, copulation, comparative study.

INTRODUCTION

The dwarf spider *O. gibbosus* (Blackwall, 1841) is a rather rare spider in Flanders. It can be found in wet to very wet habitats, such as oligo- and mesotrophic alder carrs (De Keer & Maelfait, 1989; Alderweireldt, 1992). Besides *O. gibbosus*, other representatives of the genus *Oedothorax* are present in Belgium : *O. apicatus* (Blackwall, 1850), *O. agrestis* (Blackwall, 1853), *O. fuscus* (Blackwall, 1834) and *O. retusus* (Westring, 1851). *O. agrestis* is even more rare than *O. gibbosus*, and is also restricted to wet to very wet habitats (De keer & Maelfait, 1989). The other three species on the other hand are among the most common spider species in Belgium. They frequently occur in enormous numbers in various types of habitats (Alderweireldt, 1992).

Only one representative of the genus *Oedothorax*, *O. gibbosus*, is characterised by male dimorphism (De keer & Maelfait, 1989). The genetically dominant (Maelfait et al., 1990; Vanacker et al., 2001b) *gibbosus* morph possesses a large protuberance on the last third of the carapace, preceded by a hairy groove. The *tuberosus* morph on the other hand does not possess such a groove and its carapace is smooth and convex.

In *O. fuscus* the highest point of the male carapace is situated in the anterior part, and there is typically a pale region in the middle of the abdomen of the female. On the clypeus there are two long hairs (Alderweireldt, 1992). This last feature is also typical for *O. agrestis* males. However, the cephalic part of an *O. agrestis* male is very similar to that of the *tuberosus* morph of *O. gibbosus*; the posterior part of the carapace of *tuberosus* is slightly higher than that of *O. agrestis* males. In *O. retusus*, the anterior part of the carapace is strongly raised and there is only one hair on the clypeus (Alderweireldt, 1992). On both sides of the carapace, there are grooves with pores (Schaible et al., 1986). *O. apicatus* also possesses lateral grooves (Schaible et al., 1986) and only one hair on the clypeus (Alderweireldt, 1992). Typical for this species is the presence of a narrow protuberance on the highest part of the male carapace (Schaible et al., 1986; Roberts, 1987).

Dwarf spiders of the genus *Oedothorax* are approximately 3 mm long and the females are bigger than the males. Most of the time, copulation takes place upside down in the web. In this way, the male finds itself above the female and inserts a palp in the epigyne. Each time a small quantity of sperm is transmitted from the palp into the female spermathecae, by means of a hydraulic pumping system of the haematodoch-bladder (Foelix, 1996). In many cases, copulation is preceded by courtship, which can have a gustatorial character and can be considered as 'nuptial feeding'. The term 'nuptial feeding' refers to any form of offering food by the male to the female, during or immediately after the courtship or (and) copulation (Vahed, 1998). The phenomenon of secretion in cephalothoracic protrusions is not an exclusive character of the family Linyphiidae. Such secretions are also found in other

families, for example in *Argyrodes antipodiana* O.P.-Cambridge, 1880 (Theridiidae) (Whithouse, 1987).

Vanacker et al. (in press) mentioned several interspecific gustatorial courtships by a *gibbosus* male, and a male or female of the closely related species *O. fuscus.* These interspecific interactions suggest that the hairy groove in the *gibbosus* male morph is a nuptial feeding device possibly under the influence of sexual selection. The interspecific interactions can possibly be interpreted as 'robbings' of the nuptial feeding (Vanacker et al., in press). Histological data (Vanacker, unpubl.) confirm that *gibbosus* males secrete a nuptial gift in their groove; the hunch of *gibbosus* is filled with gland cells with different kinds of secretions.

Here we describe intraspecific courtship behaviour in the different *Oedothorax* species. Because only *O. apicatus* males have a clearly distinct protuberance on the carapace comparable with the hunch of *gibbosus* males, we assume that the possibility of the occurrence of a gustatorial courtship is higher in *O. apicatus* than in *O. fuscus, O. retusus* and *O. apicatus.*

We also describe copulation behaviour of the different *Oedothorax* species. The copulation of *Erigone atra* (Blackwall, 1833) is also taken into account, because *E. atra* is closely related to the *Oedothorax* species according to the phylogenetic trees of Hormiga (2000). *E. atra* is, like *O. fuscus*, *O. retusus* and *O. apicatus*, a very common species of open habitats. It can be supposed to be very active to increase its chances of survival in the open field by evading natural predators. This active behaviour can have consequences for the duration of copulation. *O. gibbosus* and *O. agrestis* on the other hand are rare species, which are restricted to a very specific habitat. Such species probably have the opportunity to copulate more slowly and for longer.

Here, we investigate the hypothesis about the occurrence of gustatorial courtship as well as the hypothesis about copulation duration. Also the function of the web during courtship and copulation is described. The literature about the courtship and copulation behaviour of other spider species is reviewed as well.

MATERIALS AND METHODS

The dwarf spiders were captured manually. *O. gibbosus* spiders were caught in the nature reserve Het Walenbos at Tielt-Winge, 30-km northeast of Brussels. Representatives of *O. fuscus* and *O. retusus* came from the military domain de Yzermonding at Lombardsijde. *O. apicatus* spiders were from the first generation of spiders coming from the same domain. The *O. agrestis* spiders were caught in Het Krawaalbos at Asse. The *E. atra* spiders were collected in the nature reserve De Westhoek at De Panne.

To keep the collected spiders alive as well as to breed them, the spiders were put into separate plastic cups (diameter : 4 cm and height : 2.5 cm), with a bottom of plaster and a piece of moss. Young animals were fed every two days with four springtails (*Isotoma spec.* among others). After the second moult the spiders received each time three fruit flies. At the same time three drops of water were added to maintain a relative humidity near 100%. The spiders were kept in a climatic chamber at a temperature of circa 20˚C and a photoperiod L :D of 16 :8. We opted for 20˚C because this is the best temperature to rear this dwarf spider species (Alderweireldt & De Keer, 1988; Vanacker et al., 2001a).

Copulations were observed with a WILD-binocular dissecting microscope and a cold light source. Each couple was observed for at least one hour and, in case of copulation until at least half an hour after the copulation. To take pictures of the courtship and copulation of *Oedothorax gibbosus* during our observations, we used a digital camera (Nikon).

We observed 81 copulations of *O. gibbosus*, 16 of *O. fuscus*, 22 of *O. retusus*, 24 of *O. apicatus,* 19 of *O. agrestis* and 12 of *E. atra.* Table 1 gives a complete survey of the total number of gustatorial courtships and copulations that were observed for each species.

TABLE 1

The observed numbers of courtships and copulations in five *Oedothorax* species and *Erigone atra.*

Species	Gustatorial courtship	Incomplete copulation	Complete copulation	Studied couples	Couples with 1 copulation	Couples without copulations	Couples with multiple mating
O. gibbosus							
gibbosus male	41	23	16	28	18	4	6
tuberosus male	not observed	23	19	98	37	59	2
both males	41	46	35	126	55	63	8
O. fuscus	not observed	6	10	26	13	12	1
O. retusus	not observed	4	18	24	13	7	4
O. apicatus	18 'mini- courtships'	8	16	27	8	12	7
O. agrestis	not observed	6	13	13	9	0	4
E. atra	not observed	not observed	12	32	0	20	12

RESULTS

Oedothorax gibbosus (Blackwall, 1841)

The reproductive behaviour of *O. gibbosus* is first of all characterised by a pronounced courtship behaviour, which contains two phases. During the first phase the male approaches the female, both of the partners pump with the abdomen, the male cleans its palps or does up- and down movements with the forelegs, and some males make a specific dance to seduce the female. Such a dance

consists of movements in '8' or '0' forms, which they execute while approaching the female.

During the second phase, physical contact is made between both partners. In the *gibbosus* morph the second phase is characterised by a gustatorial courtship (HEINEMANN, 1998) (Table 3). The female brings her chelicerae into the hairy groove and takes up the secretions (Fig. 1).

Such a gustatorial courtship continues on average 9.53 ± 8.68 min (n=19) (Table 2). During the second phase of the courtship, the couple gets into copulation position and the male tries to insert a palp. Courtship behaviour of the *tuberosus* morph was also observed. Because this morph does not possess a groove that is filled with a secretion, this courtship is not gustatorial. *Tuberosus* can thus only perform the aforementioned courtship acts of the first phase. Most of the observed couples courted, but in some cases, they immediately started to copulate.

Mostly the copulation itself took place upside down in the web in such a manner that the male was above the female (Fig. 2). The web has an important function in reproduction of this dwarf spider not only during gustatorial courtship, but also during normal courtship and copulation, and probably also for the distribution of contact pheromones. When copulation starts, the female chelicerae mostly stop making contact with the groove of her partner. Sometimes the transition from courtship into copulation proceeds fluently; sometimes there are interruptions. In some cases the male is dragged by the female, while its palp is still inserted. Other positions are also possible : on the bottom of the cup, on the moss or against the wall.

Fig. 1. – Gustatorial courtship of *Oedothorax gibbosus* (female left, *gibbosus* male right). Note that the picture is turned 180˚ to be more clear; the normal courtship posture is male as well as female upside down, male above female.

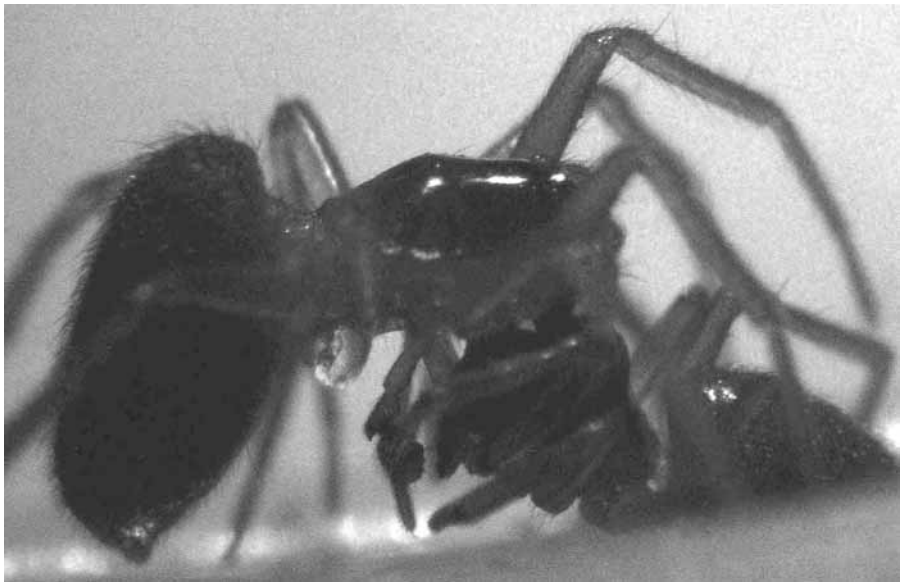

Fig. 2. – Copulation of *Oedothorax gibbosus* (*gibbosus* male right, female left). The haematodoch-bladder is also visible in the picture; the normal courtship posture is male as well as female upside-down, male above female.

During an insertion, sperm is pumped into the epigyne by means of swelling and shrinking of the haematodoch, a bladder that is part of the male palp. Insertion of the palps occurs successively; a copulation with both palps is called a 'complete copulation', while if only one insertion takes place we refer to an 'incomplete copulation' (HEINEMANN, 1998). Complete as well as incomplete copulations occur in this spider species; we observed 46 incomplete and 35 complete copulations (Table 1).

In *O. gibbosus*, one insertion takes on average about 34.05 ± 1.68 min and the second insertion about 33.39 ± 0.09 min (Table 2). In eight studied couples 'multiple mating' was noticed.

TABLE 2

Duration of courtship and copulation of *Oedothorax* species.

	Duration of courtship	Duration of copulation
Oedothorax gibbosus	9.53 ± 8.68 min (n=19) (gustatorial courtship)	1st palp : $\bar{x}$ = 34.05 ± 1.68 min, n= 81; 2nd palp : $\bar{x}$ = 33.39 ± 0.09 min, n= 35
Oedothorax fuscus	not observed	1st palp : $\bar{x}$ = 4.09 ± 1.78 min, n= 16; 2nd palp : $\bar{x}$ = 6.60 ± 0.96 min, n= 10
Oedothorax retusus	a few seconds	1st palp : $\bar{x}$ = 4.09 ± 0.97 min, n= 22; 2nd palp : $\bar{x}$ =2.61 ± 0.61 min, n=18
Oedothorax apicatus	a few seconds (‘mini-courtships’)	1st palp : $\bar{x}$ = 5.67 ± 1.74 min, n= 24; 2nd palp : $\bar{x}$ = 7.63 ± 1.41 min, n= 16
Oedothorax agrestis	not observed	1st palp : $\bar{x}$= 6.95 ± 1.81 min, n= 19; 2nd palp : $\bar{x}$ = 4.31 ± 0.75 min, n= 13

***Oedothorax fuscus* (Blackwall, 1834)**

Besides trilling of the forelegs, *O. fuscus* performed no form of courtship behaviour in the 17 studied couples (Table 3). Copulation in this species is not preceded by a gustatorial courtship, or if it takes place it is very short. We observed 16 copulations (Table 1).

Copulation position is comparable with that of *O. gibbosus*, but copulation duration is remarkably shorter (First palp : 5.88 ± 1.78 min, n=16; Second palp : 6.60 ± 0.96 min, n=10) (Table 2). The web is also very important for copulation. *O. fuscus* spiders are usually much more active than *O. gibbosus* spiders. This could possibly explain the fact that there is no courtship and that the copulation is much shorter. Another difference between the species is that *O. gibbosus* uses its head structures only during the courtship and not while copulating, whereas the *O. fuscus* female is in contact with the male cephalic structures during copulation. The head structures of *O. fuscus* are only used to force the spider into a better position. Multiple mating was noticed in one couple.

TABLE 3

Contact of the female chelicerae with the male head structures in the five *Oedothorax* species.

Species	Contact during courtship	Contact during copulation	Type of courtship
Oedothorax gibbosus	X	/	Pronounced courtship Gustatorial courtship (*gibbosus* morph)
Oedothorax fuscus	/	X	No pronounced courtship No gustatorial courtship observed
Oedothorax retusus	/	in some cases	Pronounced courtship No gustatorial courtship observed
Oedothorax agrestis	/	in some cases	Distinct courtship No gustatorial courtship observed
Oedothorax apicatus	X	X (hunch)	Mini-courtships No gustatorial courtship observed

***Oedothorax retusus* (Westring, 1851)**

In this species there is, in most cases, a courtship (Table 3). The most common courtship acts are trembling with the first pair of legs, plucking on the web with the forelegs, cleaning the palps, etc. In one case, the male executed some kind of dance; by sinking a few times through its legs, alternating with raising itself, it worked itself up and down. This behaviour took place while it stood erect in the web, and was combined with trembling of the opisthosome. In the second phase of the courtship, both partners touched each other with the forelegs and put themselves into copulation position. Meanwhile the male attempted to insert a palp.

The copulation itself usually takes place upside down under the web, although one copulation took place on the bottom and another one against the wall of the cup. The position is comparable with that of *O. gibbosus*. Like *O. fuscus*, copulation duration of *O. retusus* is much shorter than that of *O. gibbosus* (First palp : 4.09 ± 0.97 min, n=22; Second palp : 2.61 ± 0.61 min, n=18) (Table 2). In four studied couples, multiple mating was noticed. In most cases, the female mouthparts were situated above the head structures of the male during copulation.

***Oedothorax agrestis* (Blackwall, 1851)**

In *O. agrestis* no obvious courtship behaviour was noticed (Table 3). The copulation position was comparable with that of *O. gibbosus*. Although this species behaves rather calmly, the copulation duration was remarkably shorter than that of *O. gibbosus* (First palp : 6.95 ± 1.81 min, n=19; Second palp : 4.31 ± 0.75 min,

n=13) (Table 2). One couple copulated twice. These results are in a way different from those formulated by SCHLEGELMILCH (1974) who mentioned an average duration of insertion of 7.5 min.

According to SCHAIBLE et al. (1986), there is no contact between the female chelicerae and the male head structures. In one case we observed, however, that the female mouthparts were situated above the male head structures. In the other cases there was no contact during the copulation. In four studied couples, multiple mating was noticed.

***Oedothorax apicatus* (Blackwall, 1850)**

This representative of the *Oedothorax* genus is remarkable for its short, nervous and fierce copulation (First palp : 5.67 ± 1.74 min, n=24; Second palp : 7.63 ± 1.41 min, n=16) (Table 2). In many cases the female held the protuberance (hunch) of the male between the chelicerae (seven observations). Both partners trilled a lot while copulating and the female usually dragged the male violently and over long distances.

The presence of the hunch as well as the solid stitch between the palp and the epigyne, assures that the copulation is not interrupted. The copulation position is strongly comparable with that of *O. gibbosus*, with exception of the continuing contact between hunch and chelicerae. Because of the nervous copulation, the function of the web is apparently of less importance for copulation in *O. apicatus*.

Analogously with the courtship of other more active spiders, such as *O. fuscus* and *O. retusus*, the courtship of *O. apicatus* is very short and nervous. Moreover, several times so-called "mini-courtships" were executed; the female held the male hunch by means of her chelicerae, in addition to which the female touched the head of the male with the first pair of legs (Table 3). During this "mini-courtship", however, the female did not make sucking or biting movements with the mouth, as was the case in *O. gibbosus*. In seven studied couples 'multiple mating' was noticed.

***Erigone atra* (Blackwall, 1833)**

An elaborate courtship was not observed in *E. atra*. While approaching the female, the male moved its palps up and down, trembled briefly with its forelegs and pumped a single time with its abdomen.

The copulation is different from that of the *Oedothorax* species : instead of two continuous insertions we observed, in this case here, series of very short insertions (9.81 ± 5.32 min, n=93), which could be linked to one another to form longer periods. During such a series, the male inserts one pedipalp, pumps once (haematodoch swells and shrinks one time), removes the palp, cleans it, inserts the other (in some cases the same) palp, etc. The aforementioned longer periods continued for about 83.42 ± 5.99 min (n=12) and embraced, in four couples seven, in one couple eight, in six couples nine and in the other couple eleven series of insertions.

DISCUSSION

The majority of the erigonine males carry head structures of various kinds : elevations, accretions, notches, grooves and hair fields. SCHAIBLE & GACK (1987) mentioned that all species possessing such structures, have in common that the chelicerae of the female makes contact with the male head structures during copulation. This statement appears not to be true; with *E. atra*, for example, there was no contact observed, neither during courtship, nor during copulation (SCHAIBLE et al., 1986). SCHAIBLE et al. (1986) did not observe any contact of the female mouthparts with the male head structures. Our observations, however, showed one case in which the female chelicerae were above the head structures of the male.

Although many dwarf spiders court before copulating, we found mention in the literature of few dwarf spider species that make physical contact during courtship : *Baryphyma pratense* (Blackwall, 1861) and *Ceratinella brevipes* (Westring, 1851) (SCHLEGELMILCH, 1974; SCHAIBLE et al., 1986). Other spider species which make chelicerал contact during courtship are *Atypus muralis* Bertkau, 1890 and *Atypus piceus* (Sulzer, 1776) (Atypidae), *Avicularia avicularia* (Linnaeus, 1758), *Grammostola mollicoma* (Ausserer, 1875) and *Phormictopus canceridis* (Latreille, 1806) (Therophosidae), *Scytodes thoracica* (Latreille, 1802) and *Scytodes velutina* Heineken & Lowe, 1832 (Scytodidae), *Holocnemus pluchei* (Scopoli, 1763), *Pholcus phalangioides* (Fuesslin, 1775) (Pholcidae), *Segestria bavarica* C. L. Koch, 1843, *Segestria florentina* (Rossi, 1790) and *Segestria senoculatata* (Linnaeus, 1758) (Segestriidae), *Pachygnatha clercki* Sundevall, 1823, *Pachygnatha degeeri* (Sundevall, 1830) and *Pachygnatha listeri* (Sundevall, 1830), *Tetragnatha extensa* (Linnaeus, 1758), *Tetragnatha montana* Simon, 1874 and *Tetragnatha nigrita* (Lendl, 1886) (Tetragnathidae), *Nigma walckenaeri* (Roewer, 1951) (Dictynidae), *Clubiona germanica* (Thorell, 1871) and *Clubiona terrestris* (Westring, 1851) (Clubionidae) (HUBER, 1998).

The copulation duration of *Oedothorax gibbosus* is comparable with that of *Ceratinella brevipes* (Westring, 1851), *Diplocephalus latifrons* (O.P.-Cambridge, 1863), *Gonatium rubellum* (Blackwall, 1841) (SCHLEGELMILCH, 1974; SCHAIBLE et al., 1986). In other spider families, for example *Dysdera erythrina* (Walckenaer, 1802) (Dysderidae), *Oonop placidus* Dalmas, 1916 (Oonopidae), *Pisaura mirabilis* (Clerck, 1757) (Pisauridae), *Xysticus cristatus* (Clerck, 1757) (Thomisidae) and *Heliophanus cypreus* (Walckenaer, 1802) (Salticidae) have similar copulation duration to *Oedothorax gibbosus* (HUBER, 1998).

Our results show that, within the genus *Oedothorax*, *O. gibbosus* differs from its sister species by executing a longer courtship, certainly in the case of the *gibbosus* male, as well as a longer copulation. We observed gustatorial courtship only in *O. gibbosus*, although we also observed "mini-courtships" in *O. apicatus*.

The *gibbosus* morph has a hairy groove in which a nuptial gift and/or pheromones are secreted (VANACKER et al., in press). The *tuberosus* morph, on the contrary, has no distinct head structures. They have few cephalic gland

cells (VANACKER, unpublished results) and probably this is also the case in the males of *Oedothorax* species without distinct cephalic structures. *O. apicatus* can be regarded as taking an intermediate position : an *O. apicatus* male is characterised by one narrow hunch on its cephalothorax. The possible occurrence of a few gland cells could explain the appearance of the "mini-courtships" in this species. The results of MEIJER (1976) indirectly confirm this hypothesis; the author found remnants of secretions on spiders that were caught in pitfalls, including on *O. apicatus*. This article also mentions possible secretions in *Troxochrus scabriculus* (Westring, 1851), which is also known for its male dimorphism. Further histological and TEM research will study the occurrence of such cells.

Probably most erigonine males possess gland cells (some species more than others). The gustatorial courtship is most pronounced in *O. gibbosus* (probably linked to the male dimorphism of this species), but the mini-courtships of *O. apicatus* also have in most cases a 'gustatorial' character (MEIJER, 1976). It is possible that the biting movements only appear in *O. gibbosus*, because the substance, in that species, is secreted in a groove. In this way, heavier sucking movements are necessary for the female to reach the secretion. Another possibility is that because of this groove, the female first places some saliva into the groove and then sucks up (SCHAIBLE & GACK, 1987). We have observed this several times (VANACKER, unpubl.). Because the possible secretions in the other *Oedothorax* species are superficial (i.e. not in a groove), females of this species do not have to execute pronounced bite and suck movements. Courtship of the kind seen in *O. apicatus* or simple contact during copulation as in *O. retusus* and *O. agrestis* could also have a gustatorial character. Gustatorial contact in these last species is not so obvious, because the contact is much shorter and the secretions are probably less. In *O. fuscus*, this contact is probably so short that it almost cannot be observed during copulation. It is also possible that there are secretions in *E. atra*. Superficial contacts between female mouthparts and male head structures to exchange secretions remain a possibility.

If we compare courtship and copulation behaviour of *Oedothorax* species with the other dwarf spiders, we can conclude that the courtship behaviour is comparable within the subfamily Erigoninae. *Oedothorax* species are characterized by a copulation of two insertions, which is typical for most dwarf spiders. This also appears in some other dwarf spider species, but there are also species whose copulations contain several series of insertions, such as *Erigone* species*, Tmeticus graminicolus*, etc. We observed in *E. atra* series of very short insertions, which can be linked to each other to form longer periods. SCHLEGELMILCH (1974) also mentioned this, but he claims an increasing duration of insertion; we found on the contrary more or less regular insertion times. The copulation duration, according to SCHLEGELMILCH (1974) is 100-150 minutes; in our results we found an average duration of 83.5 ± 7.78 min (n=12) per longer period. *Erigone dentipalpis* (Wider, 1843) and *Erigone longipalpis* (Sundevall, 1830) seem to have similar copulation behaviour to that of *E. atra* (SCHLEGELMILCH, 1978).

The copulation of many species in which the male possesses head structures, is characterised by an anchorage of the male head structures by the female chelicerae (*Hypomma bituberculatum* (Wider, 1834), *Walckenaeria corniculans* (O.P.-Cambridge, 1875) : SCHLEGELMILCH, 1974). *O. gibbosus* only shows physical contact of the female chelicerae with the groove and hunch of the *gibbosus* male, during gustatorial courtship. SCHAIBLE & GACK (1987) suggested that the function of the male head structures is to fix the copulation posture. This is probably not the most common function, because there is, for example, in most cases no retained contact between the chelicerae and the male head structures during copulation of *O. gibbosus*.

Oedothorax species have a relatively short copulation duration, excepted for *O. gibbosus*, where average copulation duration is strikingly longer than that of the other *Oedothorax* species. The longest copulation duration belongs to *Erigone atra* (50'-75'/ insertion) – but this last species has another copulation technique – and the shortest to *Tmeticus graminicolus* (5' per insertion). The extremely high copulation duration of *O. gibbosus* is very remarkable. Why, however, is such a long copulation necessary, if closely related species can achieve sperm transfer in a much shorter time? A longer copulation time leads to a higher risk of being caught by a predator. Possibly this risk is more pronounced for species living in open habitats than in more rare ones. There is for example no specific natural predator of *O. gibbosus* in the studied population in the public nature reserve Het Walenbos; the lycosid spider *Pirata hygrophilus* Thorell, 1872 occurs at high density but does not predate on *O. gibbosus* (VANACKER, unpubl.). That the copulation of the rare *O. agrestis* is short is in disagreement with the above-mentioned hypothesis. However, this species behaves rather calmly, which is comparable to the activity of the also rare *O. gibbosus.*

Multiple mating occurs very frequently in *Oedothorax* and *Erigone*. The number of couples that performed multiple mating in *O. gibbosus* was not very high in the described experiment, but in other experiments with longer observation periods multiple mating was also very frequent in both male morphs of *O. gibbosus* (VANACKER, unpubl.). The web has an important function in the reproduction of *Oedothorax* spiders. It seems reasonable to assume the importance of the web in reproduction decreases with the activity of the spiders.

The longer courtship and copulation time could also be a consequence of the occurrence of male dimorphism in this species caused by male competition and sperm competition. More ethological and genetic research, such as construction of a phylogenetic tree of *Oedothorax* on the basis of DNA, is necessary to give a decisive answer about this.

ACKNOWLEDGEMENTS

We are grateful to Domir De Bakker, Frederik Hendrickx and Dries Bonte for checking the identification of the living spiders. For catching the animals we could depend on Katrijn Deroose, Viki Vandomme, Lut Van Nieuwenhuyse, Domir De Bakker and Léon Baert. Danny Vanacker has a PhD grant from the Foundation for Scientific Research – Flanders.

REFERENCES

ALDERWEIRELDT, M. & R. DE KEER (1988). Comparison of the life cycle history of three *Oedothorax*- species (Araneae, Linyphiidae) in relation to laboratory observations. *XI. Europäisches Arachnologisches Colloquium, Comptes Rendus du Xiieme Colloque d'Arachnologie* : 169-177.

ALDERWEIRELDT, M. (1992). Determinatieproblematiek van de zustersoorten van het genus *Oedothorax* (Araneae, Linyphiidae). *Newsbr. Belg. Arachnol. Ver.*, 9 : 19-26.

DE KEER, R. & J.-P. MAELFAIT (1989). Ecological backgrounds for the distribution patterns of *Oedothorax fuscus* (Blackwall) and *Oedothorax gibbosus* (Blackwall) (Erigoninae, Araneae). *Verhandelingen van het symposium "Invertebraten van België"* : 189-192.

FOELIX, R.F. (1996). *Biology of spiders*, 2nd Ed. Oxford University Press, Oxford.

HEINEMANN, S. (1998)**.** Männchendimorphismus bei *Oedothorax gibbosus* (Blackwall) (Araneae : Linyphiidae) : Morphometrie und Verhaltensuntersuchungen. *Diplomarbeit Rheinischen Friedrich-Wilhelms-Universität Bonn* (60 pp).

HORMIGA, G. (2000). Higher level phylogenetics of Erigonine spiders (Araneae, Linyphiidae, Erigoninae). Smithsonian Institution Press, Washington DC (160 pp).

HUBER, B.A. (1998). Spider reproductive behaviour : a review of Gerhardt's work from 1911-1933, with implications for sexual selection. *Bull. Br. Arachnol. Soc.*, 11(3) : 81-91.

MAELFAIT, J.-P., R. DE KEER & L. DE MEESTER (1990).Genetic background of the polymorphism in *Oedothorax gibbosus* (BLACKWALL) (Linyphiidae, Araneae). *Rev. Arachnol.*, 9(2) : 29-34

MEIJER, J. (1976). A glandular secretion in the ocular area of certain erigonine spiders (Araneae, Linyphiidae). *Bull. Br. Arachnol. Soc.* 3 (9) : 251-252.

ROBERTS, M.J. (1987). *Spiders of Britain and Ireland, 2 : Linyphiidae*. Harley Books, Colchester.

SCHAIBLE, U. & C. GACK (1987). Zur Morphologie, Histologie and biologischen Bedeutung der Kopfstrukturen einiger Arten de Gattung *Diplocephalus* (Araneida, Linyphiidae, Erigoninae). *Verh. Naturwiss. Ver. Hamburg,* 29 : 171-180.

SCHAIBLE, U., C. GACK & H.F. PAULUS (1986). Zur Morphologie, Histologie und biologischen Bedeutung der Kopfstrukturen männlicher Zwergspinnen (Linyphiidae : Erigoninae). *Zoologische Jahrbücher, Abteilung für Systematik, Ökologie und Geographie der Tiere (Jena)*,113 : 389-408.

SCHLEGELMILCH, B. (1974). *Zur biologischen bedeutung der kopffortsätze bei Zwergspinnenmännchen (Micry-phantidae)*. Diplomarbeit Univ. Freiburg (98 pp).

VAHED, K. (1998). The function of nuptial feeding in insects : a review of empirical studies. *Biol. Rev.*, 73 : 43-78.

VANACKER, D., J.-P. MAELFAIT & E. BYTTEBIER (2001a). Het effect van temperatuur op de ontwikkeling van de dwergspin *Oedothorax gibbosus* (Blackwall, 1841) (Erigoninae, Araneae) met speciale aandacht voor het mannelijk dimorfisme. *Nwsbr. Belg. Arachnol. Ver.* 16 (1) : 6-19.

VANACKER, D., J.-P. MAELFAIT & L. BAERT (2001b). The male dimorphism in the dwarf spider *Oedothorax gibbosus* (BLACKWALL, 1841) (*Erigoninae, Linyphiidae, Araneae) :* results of laboratory rearing experiments. *Belg. J. Zool.,* 131 *(Supplement 2) :* 39-44.

VANACKER, D., L. MAES, S. PARDO, F. HENDRICKX & J.-P. MAELFAIT (2003). Is the hairy groove in the gibbosus male morph of *Oedothorax gibbosus* (Blackwall) a nuptial feeding device? *J. Arachnol*. (in press).

WHITEHOUSE, M.E.A. (1987). The external structural detail of the protrusions on the cephalothorax of male *Argyrodes antipodiana* (Theridiidae). *Bull. Br. Arachnol. Soc.*, 7(5) :142-144.

Belg. J. Zool., 134 (supplement 1) : 37-40 March 2004

Conspicuous body coloration and predation risk in damselflies : are andromorphs easier to detect than gynomorphs ?

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ABSTRACT. The coexistence of multiple female colour morphs in damselflies remains poorly understood. Typically, one of the female morphs is coloured like the male (andromorph), while the other morphs are not (gynomorphs). Andromorphs, by resembling males, are thought to benefit from avoiding male harassment. Some authors have proposed that this benefit is offset by a higher probability of detection for andromorphs compared to gynomorphs owing to differences in body colouration. We experimentally tested detectabilities of the different female colour morphs using human observers as model predators. In contrast to expectation, detection probabilities for andromorphs and gynomorphs were equal. We discuss the use of survival probabilities to test for differences in predation rate between female morphs and consider whether human predators are representative models for the natural predator guild of the studied damselfly.

KEY WORDS : colour polymorphism, predation, detection, Odonata.

INTRODUCTION

Despite many studies, the evolution and maintenance of female-limited colour polymorphism in damselflies remains puzzling (e.g. Fincke, 1994; Cordero et al., 1998; Andrés et al., 2000; Sherratt, 2001; Andrés & Cordero, 2001). Typically, one of the female morphs (andromorph) is coloured, and in some species also patterned like the male, while the additional morphs (gynomorphs) are not (e.g. Corbet, 1999). Andromorphs, by resembling males, are thought to have a selective advantage through avoiding male harassment (e.g. Johnson, 1975; Robertson, 1985; Cordero et al., 1998; Sherratt, 2001). Some researchers proposed that this benefit might be offset by a higher probability of detection and predation by visual predators of the conspicuously coloured andromorphs compared to the cryptic gynomorphs (Johnson, 1975; Robertson, 1985).

To the best of our knowledge, no study has examined directly whether female morphs differ in probability of detection as a result of differences in body colouration. Forbes (1994) did not find any differential predation by dragonflies on copulating females of the damselfly, *Enallagma boreale* (Selys, 1875). However, in his experiment either the copulating male or the female was alive, wherefore it cannot be excluded that the probability of detection was influenced by the behaviour of the animals under study. Further, because only predation on mating pairs was recorded, it remains inconclusive whether female morphs on their own differ in susceptibility.

Several researchers have studied the potential cost of predation by comparing survival between morphs in the field (e.g. Forsman & Appelqvist, 1999 for an example on colour polymorphic grasshoppers). Results from field studies using traditional capture-mark-recapture models (Seber, 1982) showed equal life spans for andro- and gynomorphs (e.g. Thompson, 1989; Fincke, 1994; Cordero et al., 1998). Recent studies using advanced capture-mark-recapture models (Lebreton et al., 1992), allowing separate estimation of survival and recapture rates, also did not reveal morph-specific differences in survival (Van Gossum, unpubl.; Andrés & Cordero, 2001). However, survival probabilities are not predictive if it comes to examining morph differences in probability of detection or predation and, therefore, should not be used to test the hypothesis by Johnson (1975) and Robertson (1985) (see Discussion and Fig. 1).

In the present study, we experimentally test the hypothesis that body colouration makes andromorphs easier to detect than gynomorphs in the damselfly, *Ischnura elegans* (Vander Linden, 1820). We further discuss the use of survival rates to assess differences in predation and detection probabilities between female colour morphs.

MATERIAL AND METHODS

The common damselfly *I. elegans* exhibits three mature female morphs, one andromorph and two gynomorphs (Parr, 1965). Andromorphs resemble the males completely in body coloration and pattern. Differences between gynomorphs are restricted to the absence of black humeral stripes on the thorax in one morph, and slight differences in the coloration of the pale parts of the

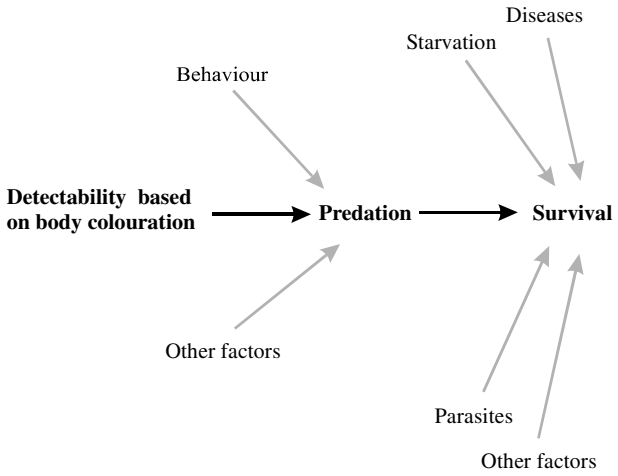

Fig. 1. – Factors that may induce survival differences between female colour morphs. The black arrows represent the hypothesised pathway following JOHNSON (1975) and ROBERTSON (1985) to explain a lower survival for the conspicuously coloured andromorphs compared to the more cryptic gynomorphs. The first step of the hypothesis was tested in the present article. The grey arrows indicate other, potentially confounding, factors that may contribute to selective predation and/or survival but that were not considered by JOHNSON (1975) and ROBERTSON (1985).

thorax. Gynomorphs were treated as one group in this study.

We performed experiments with twenty-two naïve human observers (model predators) and dead damselflies in "De Biotuin" (Belgium, Antwerp) on 2 July 1999 between 0900 and 1600h. Experimental damselflies were collected from a population in Niel (Belgium) (where both colour morphs are abundant) the day before the experiment. To exclude behavioural differences between morphs, damselflies were killed in a bowl with chloroform one hour before the experiment. This allowed the explicit testing of differences in probability of detection based on colour differences between female morphs. The experiment was conducted at a pond with a small resident natural population of the study species. Hence, we consider it reasonable to assume that the background applied in the experiment was relevant to examine differential detectability between female morphs. Human observers were asked to walk along thirteen stops each marked with a wooden stick while accompanied by one of the authors (HVG). The author always preceded the human observer to arrive before the observer at the next stick. A few times the author needed to chase away a foraging damselfly of the resident population, an action always done without notice by the human observer. The sticks guided the observers along the "predation" sites. At each stick, within a radius of 0.5m, one dead andro- and one dead gynomorph were glued onto stems or leaves (using a drop of instant glue on the legs and at the abdomen of the animals). Care was taken to position animals in a natural and random way. Therefore, we selected two comparable locations at a stick before randomly attaching the two morphs. Damselflies were glued on positions between 20-90cm height in the vegetation in an upright position.

A single human observer was asked to squat down at each stick and to screen the area for small insects without touching the vegetation. Screening was confined within a 0.5m radius of the stick. Preliminary tests showed that when the radius was larger, search time increased considerably, and/or the human observers failed to find the animals. After detection of one damselfly or after a maximum search time of thirty seconds, the observer was asked to move on to the next stick and to repeat the search until all sticks were visited. Human observers only knew they had to search for dead insects and were not provided with any further information concerning species, colour patterns, number of animals hidden or aim of the study. Consequently, during the first encounter the human observer could be regarded as inexperienced with the presented prey species. In field conditions, however, predators are often experienced and search actively for a particular prey species (e.g. KREBS & DAVIES, 1997). Thus, repeating the search sessions using the same human observer over several sticks mimics to some extent the increasing experience of natural predators hunting for prey.

To test for differences in detectability between morphs we performed a repeated measure logistic regression with the detected morph (gynomorphs=0; andromorph=1) as dependent variable. Since subsequent observations of the same test person are not statistically independent, observer was added as repeated measure. Several covariance structures were modelled, but they all gave identical results. To account for differences in morph detectability among sticks, stick was added to the model as a random variable (GLIMMIX macro SAS 8.02; LITTELL et al., 1996). We tested 1) whether, in general, andromorphs are more conspicuous than gynomorphs, 2) whether this conspicuousness increases during the course of the experiment (stick number as fixed effect), and 3) whether morph-specific encounter experience influences detectability (cumulative morph-specific previous encounters as fixed effect). Correct degrees of freedom were obtained by the satterthwaite formula.

RESULTS

In the majority (75%) of the cases a damselfly was detected at a stick (N=286). Mean detectability over all encounters was equal for andromorphs and gynomorphs ($t=1.20$; $df=10.6$; $p=0.26$). The detectability of andromorphs did not change with increasing number of sticks visited ($F_{1, 9.55=}0.87$; $p=0.37$), neither did the detectability of andromorphs change with the number of previous encounters with that specific morph ($F_{1, 68=}0.60$; $p=0.44$).

DISCUSSION

This study is the first that experimentally tests and rejects the hypothesis that andromorph damselflies are easier to detect by a predator (human observer) due to their body colouration. Our observations support the hypothesis that survival is equal in the two female colour morphs, and this should be interpreted as evidence against selective detection.

However, detection in living damselflies can also be induced by other factors than colour alone (Fig. 1). Indeed, andro- and gynomorphs also differ in activity pat-

terns (e.g. Van Gossum et al., 2001), mating avoidance tactics (e.g. Robertson, 1985; Van Gossum et al., 2001; Sirot et al., 2003) and habitat use (e.g. Forbes et al., 1995; Van Gossum et al., 2001). Gynomorphs occupy less open habitat and often fly away when a male approaches, while andromorphs use more open habitat, do not fly large distances and directly face approaching males (Van Gossum et al., 2001). Evidently, such differences may lead to differences between morphs in probability of detection and/or predation. Rehfeldt (1995), for example, found that territorial damselflies were more likely to get trapped in spider webs than non-territorial ones. Therefore, the absence of differences in probability of detection may not be extrapolated to the field if other differences besides body colouration between female morphs are included.

Further, we have to question whether human predators can serve as a model for the natural predator guild (see also Bennett et al., 1994; Majerus et al., 2000). Earlier studies, nevertheless, showed that the use of human observers to test ecological questions can render very interesting insights into poorly understood mechanisms (e.g. Götmark & Hohfält, 1995; Van Damme & Van Dooren, 1999; Cuadrado et al., 2001). Predation on adult damselflies is recorded for spiders, waterstriders, wasps, robberflies, dragonflies, frogs and birds (Johnson, 1975; Parr & Parr, 1972; Rehfeldt, 1995; Stoks & De Bruyn, 1996; Corbet, 1999). Although web spiders are commonly recorded as predators (Larochelle, 1978; Rehfeldt, 1995; Cordero et al., 1998), they do not actively search for prey. Some spiders do actively search for prey (e.g. Thomisidae, Pisauridae, Salticidae) and are able to distinguish between different prey (e.g. Jackson & Li, 1998). Frogs are very common but not selective, attacking any moving object (e.g. Michiels & Dhondt 1990). Among the remaining groups, at least dragonflies and birds are known to possess excellent vision and the ability to develop a search image (e.g. Bond & Kamil, 2002; Corbet, 1999). Together, some groups of predators may be very similar to humans if it comes to detection of damselflies. Future experiments will be needed to shed more light on the possible impact of these predators.

Some researchers have used survival probabilities of morphs to study the potential cost of predation (e.g. Cordero et al., 1998). Capture-mark-recapture models, however, only generate survival probabilities, but do not provide any information on the mechanisms shaping these probabilities. Therefore, we question whether survival probabilities are informative on the probability of detection? As for other insects in general, mortality in natural damselfly populations can be caused by many factors such as predation, interactions with conspecifics, dehydration, starvation, parasites and diseases (Fig. 1; Corbet, 1999). The absence of morph-specific differences in survival implies that andro- and gynomorphs suffer equally from the sum of all these mortality factors, although there may be significant differences in the respective contributions of these factors. In other words, equal (or unequal) survival probabilities among morphs do not necessarily give information on differences in mortality due to predation or on probability on detection (see also Andrés & Cordero, 2001). That other mechanisms besides predation are at work in shaping survival in female damselflies is supported by an experiment with *I. elegans* where survival differences between female colour morphs were recorded, while cannibalism and predation were excluded (Van Gossum, 2001). Therefore, we do not favour the use of survival probabilities for examining selective pressures such as predation.

ACKNOWLEDGEMENTS

We are very grateful to Kris Lauwers, Jan Scheirs and two anonymous referees for constructive comments and suggestions on earlier drafts of the manuscript. Participants of the Summer School for Biology (1999) and students and colleagues of the University of Antwerp kindly volunteered for this study. HVG and RS are postdoctoral fellows with the Fund for Scientific Research-Flanders (FWO).

REFERENCES

Andrés, J.A. & A. Cordero (2001). Survival rates in a natural population of *Ceriagrion tenellum* (Villers) : effects of sex and female phenotype. *Ecol. Entom.*, 26 : 341-346.

Andrés, J.A., R.A. Sanchez-Guillen & A.C. Rivera (2000). Molecular evidence for selection on female color polymorphism in the damselfly *Ischnura graellsii*. *Evolution*, 54 : 2156-2161.

Bennett, A.T.D., I.C. Cuthill & K.J. Norris (1994). Sexual selection and the mismeasure of color. *Am. Nat.*, 144 : 848-860.

Bond, A.B. & A.C. Kamil. (2002). Visual predators select for crypticity and polymorphism in virtual prey. *Nature*, 415 : 609-613.

Corbet, P.S., (1999). *Dragonflies : Behaviour and ecology of Odonata.* Martins, Harley Books, Essex, UK (829 pp).

Cordero, A., S.S. Carbone & C. Utzeri (1998). Mating opportunities and mating costs are reduced in androchrome female damselflies, *Ischnura elegans* (Odonata). *Anim. Behav.*, 55 : 185-197.

Cuadrado, M., J. Martin & P. Lopez (2001). Camouflage and escape decisions in the common chameleon *Chamaeleo chamaeleon*. *Biol. J. Lin. Soc.*, 72 : 547-554.

Fincke, O.M. (1994). Female colour polymorphism in damselflies : failure to reject the null hypothesis. *Anim. Behav.*, 47 : 1249-1266.

Forbes, M.R.L. (1994). Tests of hypotheses for female-limited polymorphism in the damselfly, *Enallagma boreale* Selys. *Anim. Behav.*, 47 : 724-726.

Forbes, M.R.L., J.M.L. Richardson & R.L. Baker (1995). Frequency of female morphs is related to an index of male density in the damselfly, *Nehalennia irene* (Hagen). *Ecoscience*, 2 : 28-33.

Forsman, A. & S. Appelqvist (1999). Experimental manipulation reveals differential effects of colour pattern on survival in male and female pygmy grasshoppers. *J. Evol. Biol.*, 12 : 391-401.

Götmark, F. & A. Hohlfält (1995). Bright male plumage and predation risk in passerine birds : are males easier to detect than females? *Oikos*, 74 : 475-484.

Jackson, R.R. & D.Q. Li (1998) Prey preferences and visual discrimination ability of *Cyrba algerina*, an araneophagic jumping spider (Araneae : Salticidae) with primitive retinae. *Israel J. Zool.*, 44 : 227-242.

Johnson, C. (1975). Polymorphism and natural selection in ischnuran damselflies. *Evol. Theory*, 1 : 81-90.

Krebs, J. R. & N.B. Davies (1997). *Behavioural Ecology : An Evolutionary Approach 4th edn.* Oxford : Blackwell Science (420 pp).

Larochelle, A. (1978). Spiders as predators and prey of Odonata. *Cordulia*, 4 : 29-34.

Lebreton, J.D., K.P. Burnham, J. Clobert & D.R. Anderson (1992). Modelling survival and testing biological hypotheses using marked animals : a unified approach with case studies. *Ecol. Mon.*, 62 : 67-118.

Littell, R.C., G.A. Milliken, W.W. Stroup & R.D. Wolfinger (1996). *SAS System for mixed models.* Cary, NC : SAS Institute Inc (656 pp).

Majerus, M.E.N., C.F.A. Brunton & J. Stalker (2000). A bird's eye view of the peppered moth. *J. Evol. Biol.*, 13 : 155-159.

Michiels, N.K. & A.A. Dhondt (1990). Costs and benefits associated with oviposition site selection in the dragonfly *Sympetrum danae* (Odonata : Libellulidae). *Anim. Behav.*, 40 : 668-678.

Parr, M.J. (1965). A population study of a colony of imaginal *Ischnura elegans* (Vander Linden) (Odonata : Coenagrionidae) at Dale, Pembrokeshire. *Field Study*, 2 : 237-282.

Parr, M.J. & M. Parr (1972). Survival rates, population density and predation in the damselfly, *Ischnura elegans* (Vander Linden) (Zygoptera : Coenagrionidae). *Odonatologica*, 1 : 137-141.

Rehfeldt, G.E. (1995). *Naturliche feinde, Parasiten und Fortzplantung von Libellen.* Odonatological monographs 1, Braunschweig (154 pp).

Robertson, H.M. (1985). Female dimorphism and mating behaviour in a damselfly, *Ischnura ramburi* : females mimicking males. *Anim. Behav.*, 33 : 805-809.

Seber, G.A.F. (1982). *The estimation of animal abundance.* Charles Griffin and Company, London (274 pp).

Sherratt, T.N. (2001). The evolution of female-limited polymorphisms in damselflies : a signal detection model. *Ecol. Lett.,* 4 : 22-29.

Sirot, L.K., Brockmann, J., Marinis, C. & Muschett, G. (2003). Maintenance of a female-limited polymorphism in *Ischnura ramburi* (Zygoptera : Coenagrionidae). *Anim. Behav.*, 66 : 763-775.

Stoks, R. & L. De Bruyn (1996). Intensive feeding of the robberfly *Eutolmus rufibarbis* (Diptera Asilidae) on the damselflies *Enallagma cyathigerum* and *Lestes sponsa* (Odonata). *Bull. Annls. Soc. r. belge Ent.*, 132 : 427-431.

Thompson, D.J. (1989). Lifetime reproductive success in andromorph females of the damselfly *Coenagrion puella* (Zygoptera : Coenagrionidae). *Odonatologica*, 18 : 209-213.

Van Damme, R. & T.J.M. Van Dooren (1999). Absolute versus per unit body length speed of prey as an estimator of vulnerability to predation. *Anim. Behav.,* 57 : 347-352.

Van Gossum, H., R. Stoks & L. De Bruyn (2001). Frequency-dependent male harassment and intra-specific variation in its avoidance by females of the damselfly *Ischnura elegans*. *Behav. Ecol. Sociobiol.,* 51 : 69-75.

Van Gossum, H. (2001) *Evolutionary ecology of female colour polymorphism in a damselfly.* PhD dissertation, Universiteit Antwerpen (115 pp).

Belg. J. Zool., 134 (supplement 1) : 41-45 March 2004

Energy budgets in the simultaneously hermaphroditic pond snail, *Lymnaea stagnalis* : A trade-off between growth and reproduction during development

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ABSTRACT. Maximum lifetime reproductive success is determined by the optimal partitioning of available resources between growth, maintenance and reproduction. The main question that is addressed here is how this resource allocation occurs in the simultaneously hermaphroditic pond snail, *Lymnaea stagnalis*. Snails were either reared in groups or in isolation and were fed a standard, restricted amount of lettuce; group-reared snails were isolated when egg laying started. Snails reared in isolation seldom produce eggs. Instead, they increase growth rate and the energy invested in this growth corresponds to that invested in eggs by group-reared animals. Additionally, animals reared in isolation have larger prostate glands. Hence, when no mating partners are available, snails mainly invest in growth as well as the male function. Allocation to female reproduction only starts once copulation has taken place. These findings reveal a trade-off between growth and female reproduction. Moreover, the difference in prostate glands indicates that there is also a trade-off between investment in the male and female function. The possible existence of a sexual conflict over the onset of female reproduction is discussed.

KEY WORDS : Sex allocation, resource allocation, sexual conflict, manipulation, allohormone, snail.

INTRODUCTION

To achieve maximum lifetime reproductive success, a limited amount of resources needs to be partitioned among all life-history components in such a way that an optimal balance between growth, maintenance and reproduction is reached. To uncover the causalities that underlie these investment decisions, experimental approaches are required. Here, by interfering with the onset of female reproduction, we test how resources are allocated during development in a simultaneous hermaphrodite.

Simultaneous hermaphrodites offer unique opportunities for directly testing optimal investment of resources into male and female reproduction (Charnov, 1982; Greeff & Michiels, 1999; Pen & Weissing, 1999). Namely, a decrease in one sexual function can be directly measured as an increase in the other sexual function within the same hermaphroditic individual. We used the pond snail, *Lymnaea stagnalis* (L.), which is a simultaneous hermaphrodite that can only perform one sexual role at a time during a copulation. Therefore, for each mating encounter an individual has to decide whether to mate in the male or female role. Pond snails are usually receptive as females and are motionless when copulating in this role (Van Duivenboden & Ter Maat, 1985). The male behaviour consists of a fixed sequence of behaviours ending with penis intromission and transfer of a copious amount of sperm and seminal fluid (e.g. De Visser et al., 1994). The drive to mate as a male increases when individuals have not mated for several days (Van Duivenboden & Ter Maat, 1985). This is due to the increased amount of seminal fluid produced by and stored in the prostate gland, which is detected by the brain via a small branch of the penial nerve (De Boer et al., 1997). The area of the central nervous system that receives this information controls male reproductive behaviour and is evolutionarily conserved in gastropods (Koene et al., 2000).

Identification of the small nerve that measures the amount of seminal fluid stored in the prostate gland, allows for experimental elimination of male behaviour. When this nerve is cut the male role is eliminated, thus creating animals that reproduce in the female role only. Using this approach, De Visser et al. (1994) demonstrated that normally half of the reproductive resources that are available to the adult snail are invested in the male function. This finding agrees with theoretical predictions that, due to sperm digestion (and the resulting sexual conflict), there should be equal investment in male and female reproduction in hermaphrodites, provided that multiple mating and sperm competition occur (Greeff & Michiels, 1999). In species with separate sexes males may also invest equal amounts as females in reproduction as a result of sexual selection (Birkhead et al., 1993). Indeed, in the hermaphrodite *Lymnaea stagnalis* most of the received ejaculate is digested in the gametolytic gland. Only a very small proportion of the sperm reaches the seminal receptacle where sperm can be stored (Geraerts & Joosse, 1984). Thus, due to sperm competition and sperm digestion a large proportion of male investment is probably used to produce the copious amounts of sperm and seminal fluid.

As mentioned above, the female role during copulation mainly consists in receiving sperm. Consequently, most of the resources that are allocated to the female function

seem to go into egg production. Egg laying can be triggered by a transfer from dirty to clean water (Ter Maat et al., 1983), and is controlled by a bilateral group of neurons in the cerebral ganglia, the caudo-dorsal cells (CDCs). These neurons are electrically coupled and show synchronous bursting activity in vitro (De Vlieger et al., 1980), during which they release the egg laying hormone CDCH (Geraerts et al., 1985). Electrical in vivo recordings and stimulations confirm that these cells control egg laying (Ter Maat et al., 1986). When the egg laying hormone is experimentally introduced into the blood, egg laying behaviour follows (Ter Maat et al., 1989). Once egg laying is initiated, the eggs pass though the reproductive system, where they are provided with nutrients, mainly originating from the albumen gland.

Although *Lymnaea stagnalis* is a simultaneous hermaphrodite, development of the reproductive organs is protandric, meaning that the male reproductive organs are functional before the female ones. Additionally, it has been shown that the receipt of semen accelerates the onset of egg laying (Van Duivenboden, 1983). Without copulation, egg laying is eventually initiated, but much later. This is possibly to avoid inbreeding, because these animals can also self-fertilize, but only do so reluctantly (Van Duivenboden, 1983). Here, we investigate what happens to resource allocation during development when the female function is not initiated by copulation.

MATERIAL AND METHODS

Immature specimens of the pond snail *Lymnaea stagnalis* of equal ages and with a shell length of 15 mm were obtained from a laboratory mass culture (Van der Steen et al., 1969). They were kept at 20ºC with a light:dark cycle of 12h:12h (i.e. medium day conditions, De Visser et al., 1994). During the course of the experiment, the snails were provided with one circular disc of lettuce with a surface area of 19.6 cm^2 per day.

The 48 snails were randomly divided in two equal groups. The snails from one group were isolated in perforated plastic jars with a water volume of 460 ml. These will be referred to as the animals reared in isolation. The snails in the second group were divided equally over two perforated polyethylene boxes with a water volume of 5600 ml. This group will be referred to as group-reared. The snails were all kept in the same water tank with continuous water exchange.

After four weeks, when egg laying started in the group-reared snails, these snails were also isolated in 460 ml jars. All the jars were put randomly in the water tank. Throughout the experiment, which lasted another 25 days, egg production was monitored. The egg masses were collected at the end of the experimental period and the number of eggs was counted. All the snails, after having their body weight and length of shell determined, were anaesthetized with 50 mM $MgCl_2$ after which the shell was removed and weighed. The albumen gland and prostate gland were dissected out and their wet weights were determined. Finally, the wet and dry weights (after freeze-drying) of the remaining soft body parts were measured. Three animals died before the end of the experimental period.

The snails were fed a total of 40 lettuce rounds during the course of the experiment. Because this was less than their maximum intake all the lettuce was consumed. Hence, total consumption of each snail equalled 784 cm^2 of lettuce. The starting weight of each individual can be estimated from their initial shell height (15 mm). For this estimation, we have to calculate the dry weight density (*DWD*) of each individual by dividing the dry weight by the wet weight. Furthermore, we can calculate the shape coefficient (a) from the wet weight (W_e) and shell height (l_e) at the end of the experiment (Zonneveld & Kooijman, 1989), using the formula

$$a=(W_e)^{1/3}/l_e \qquad \text{Eq. 1}$$

With this shape coefficient, the initial wet weight (W_i) at the beginning of the experiment can be estimated for each individual using :

$$W_i=(a\, l_i)^3 \qquad \text{Eq. 2}$$

where the initial height of the shell (l_i) is 15 mm. Now, we can calculate the initial dry weight by multiplying the initial wet weight with the dry weight density. Then subtracting this value from the final dry weight (D_e) we have the estimate of the dry weight increase (dD) during the experiment :

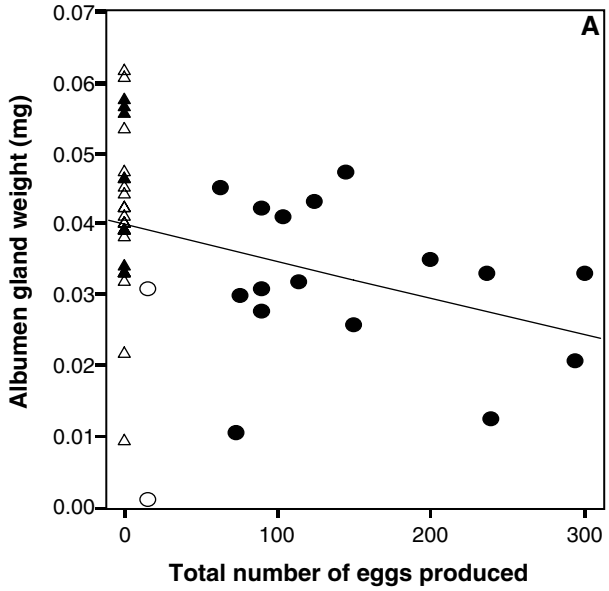
$$dD=D_e - W_i\, DWD \qquad \text{Eq. 3}$$

This allows us to compare the difference in dry weight increase between the two groups. Additionally, we know that the dry weight of one egg is 0.15 mg (Zonneveld & Kooijman, 1989). Thus, we can estimate the number of eggs that can be produced by dividing the average difference in dry weight increase between the two groups by the dry weight of one egg.

RESULTS

The animals reared in isolation had higher wet and dry weights than the group-reared animals (respectively, Wilcoxon rank sum, $p < 0.05$ and $p < 0.005$). They also had larger and heavier shells (respectively, Wilcoxon rank sum, $p < 0.005$ and $p < 0.005$). Only a few small egg masses were produced by individuals that had been reared in isolation (10.67 ± 7.54 eggs per egg mass, based on four egg masses from two snails). All the other egg masses were produced by animals that had been reared in groups, and were much larger (98.67 ± 33.98 eggs per egg mass, based on 25 egg masses from 16 snails). Table 1 shows the number of individuals that laid eggs in the two groups. The difference between the number of laying and not-laying individuals between the two groups is significant (Chi-square with Yates correction = 17.8, $p < 0.001$).

Figure 1A shows that the weight of the albumen gland was weakly and negatively correlated with the numbers of eggs that were laid (R^2=-0.17; $p < 0.05$), which shows that animals that produce many eggs deplete part of their albumen gland. There was also a trend indicating that animals that laid more eggs remained smaller (R^2=-0.40; p=0.058), which hints at a trade-off between growth and female reproduction. Figure 1B illustrates the positive correlation of the prostate gland with individual size (R^2=0.45; $p < 0.001$).



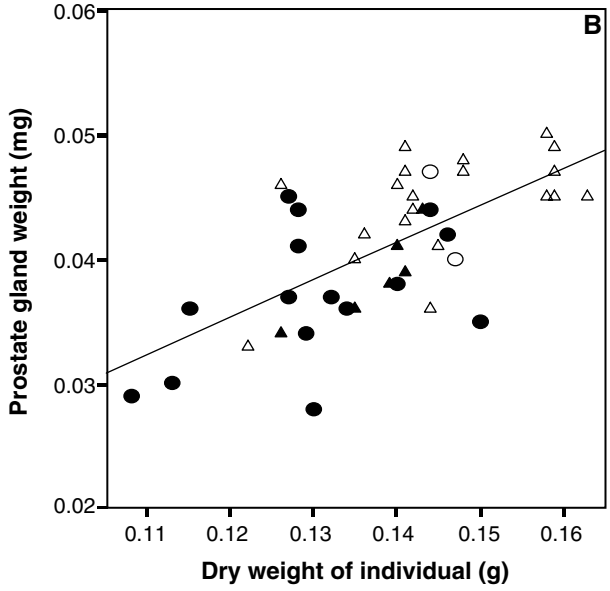


Fig. 1. – 1A. – Relationship between albumen gland weight and total number of eggs produced. The fitted line shows the negative correlation between the weight of the albumen gland and the number of eggs laid for all the individuals ($R^{2=}$-0.17; $p < 0.05$). 1B. – Relationship between prostate gland weight and dry weight of the whole animal. The fitted line shows the positive correlation between the weight of the prostate gland and the dry weight (R^2=0.45; $p < 0.001$). Closed symbols represent the animals reared in groups, open symbols represent the animals reared in isolation. Circles stand for egg laying, triangles for not-laying.

Because the gland weights are dependent on the individual's size, we corrected for dry weight in the further statistical analyses. Additionally, we divided the data according to egg laying and rearing conditions, thus resulting in four groups : 1. snails reared in isolation that laid no eggs, 2. snails reared in isolation that laid eggs (but due to the small sample size these were excluded from the statistical comparisons), 3. snails reared in groups that laid no eggs, and 4. snails reared in groups that laid eggs.

TABLE 1

Comparison of egg laying in the two treatment groups. The number of animals laying eggs and not laying eggs is shown per rearing-condition. The difference between the treatments is significant (Chi-square with Yates correction = 17.8, $p < 0.001$). The average number of eggs per egg mass (± standard deviation) is also shown; these numbers are based on 25 and 4 egg masses from, respectively, the snails reared in groups and those reared in isolation.

	Treatment	
	Group-reared	**Reared in isolation**
No egg laying	7	20
Egg laying	16	2
(eggs per egg mass)	(98.67 ± 33.98)	(10.67 ± 7.54)

As shown in Figure 2A, the lower dry weight in the group-reared animals is due to those animals that laid eggs. Their dry weight was significantly different from that of both non-laying groups (ANCOVA with post-hoc Student's t-test, $p < 0.05$). The dry weights of animals in the two non-laying groups did not differ significantly. Additionally, we can estimate the number of eggs that could have been produced with the increase in dry weight between the two groups, using equations 1 to 3. For this calculation we used the data for the group-reared animals that did not lay eggs and those that did. This resulted in an estimated difference in average dry weight increase between the two groups of 0.0140 g, which would equal the production of 93 eggs. This estimate is lower than the observed average of 149 (± 79) eggs per individual. Nonetheless, the estimated value is closer to the observed number of eggs than the 88 eggs that we would have estimated if we had only used the observed difference in dry weight between the two groups (0.0133 g).

We can also calculate how much of the total energy intake was used for female reproduction, because we know that the total consumption per individual was 784 cm^2 of lettuce and that 0.5 cm^2 of lettuce is required to produce one egg (Zonneveld, 1993). Thus, if all the energy extracted from the lettuce was put into egg production, 1568 eggs would have been produced. The observed average was 149 eggs per individual, meaning that 9.5% of the total energy intake was used for female reproduction. This is consistent with the 11.3% difference in dry weight between the two groups. Hence, the remaining energy was invested in growth, maintenance and the male function.

In Figure 2B it can be seen that the difference between the weights of the albumen glands between the different groups was entirely due to egg laying. The albumen glands of the group-reared animals that laid eggs were significantly smaller than those of both non-laying groups (ANCOVA with post-hoc Student's t-test, $p < 0.05$). Again, between the two non-laying groups there was no difference in albumen gland weights.

The prostate glands were larger in individuals reared in isolation compared to group-reared snails (Wilcoxon rank sum, $p < 0.005$). When the snails were again divided

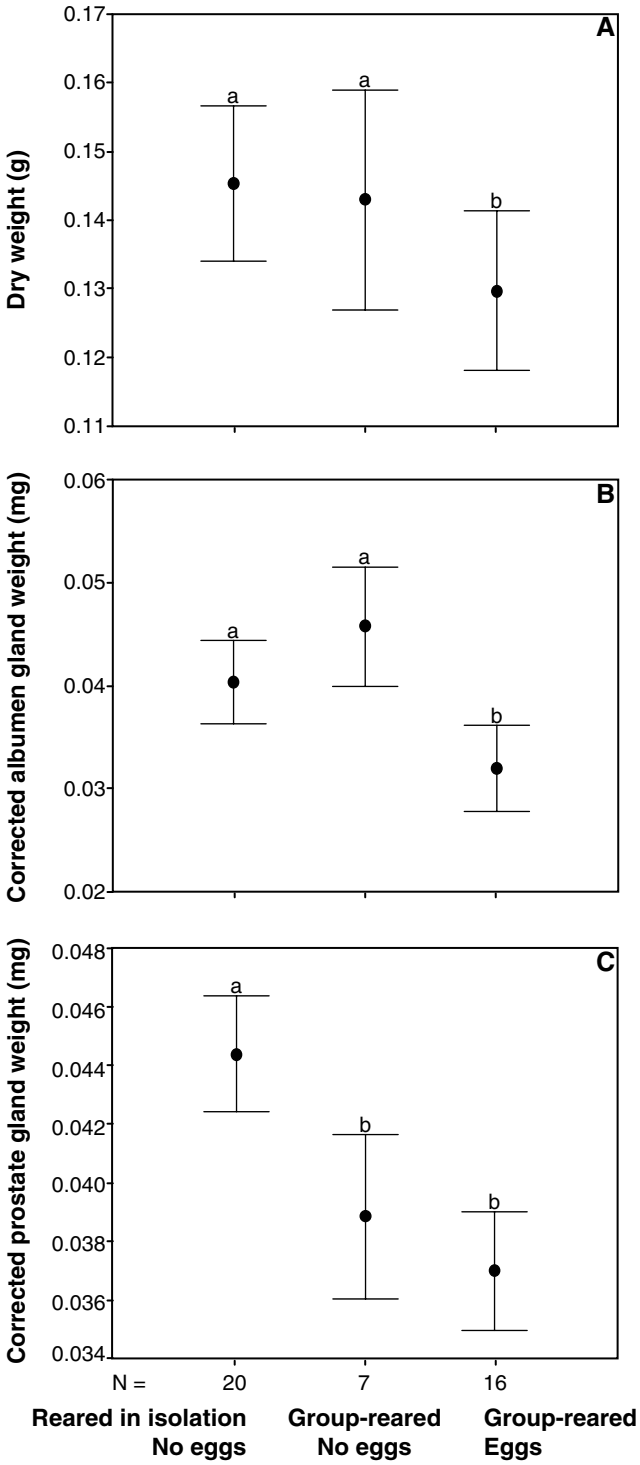


Fig. 2. – Comparison between animals grouped according to rearing condition as well as egg laying. A. The dry weight of the whole animal. B. The weight of the albumen gland corrected for dry weight of the whole animal. C. The weight of the prostate gland corrected for dry weight of the whole animal. The graphs show the mean ± standard deviation. Values for groups with different letters are significantly different (ANCOVA with post-hoc Student t-test, $p < 0.05$).

according to egg laying and rearing conditions (Fig. 2C), there was no difference between the prostate gland weights of laying and not-laying individuals of the grouped animals. But these were both significantly different from the prostate gland weights of the animals reared in isolation (ANCOVA with post-hoc Student's t-test, $p < 0.05$).

DISCUSSION

To reveal the causalities that underlie resource allocation in the pond snail, *Lymnaea stagnalis*, we made use of the delayed onset of the female function in snails reared in isolation (Van Duivenboden, 1983) and knowledge about the dynamic energy budget (Zonneveld & Kooijman, 1989). The unique advantage of using a simultaneous hermaphrodite lies in the fact that one can directly measure a decrease in one sexual function as an increase in the other sexual function (De Visser et al., 1994). Because we fed the snails a standard amount of lettuce that was below their maximum intake, consumption was known and identical in all individuals. We demonstrated that when no mating partners are available, individuals mainly invest in growth and the male function during development. Allocation of resources to female reproductive processes only starts once copulation has taken place. Our results are consistent with recent findings in the hermaphroditic bryozoan *Celleporella hyalina* (Hughes et al., 2002), and uncover a trade-off between growth and female reproduction as well as a trade-off between investment in the male and female function.

The trade-off between growth and female reproduction is evident from the differences in the dry weights and albumen gland weights between egg laying and not-laying individuals (Fig 2A and B). These results show that the smaller overall size as well as the smaller size of the albumen glands are entirely due to the production of eggs. Additionally, when estimated, the amount of energy invested in growth by the snails reared in isolation partly explains the number of eggs produced by the group-reared snails. The discrepancy between the estimated and observed number of eggs is probably due to the fact that the equations that were used to calculate the estimates are based on measurements over the life of adult snails (Zonneveld & Kooijman, 1989), while this study only looked at the start of egg laying in animals that just reached maturity. From these findings we conclude that growth is traded-off against investment in female reproduction during development.

The smaller albumen glands in the egg laying individuals (Fig. 2B) indicate that the albumen gland is partially depleted by egg laying. Although not significant, the negative trend that we found between body size and egg laying supports this. This result reveals the dynamics of the system and supports the idea that the size of the albumen gland may be an important factor that allows egg laying to be initiated; much like the role that the prostate gland plays for the male function (De Boer et al., 1997).

Besides investing more in growth, animals reared in isolation also invested more in their male function, for which the prostate gland is a measure. The difference between the groups in the development of the prostate glands, shows that there is a trade-off between the male and female function during development (Fig. 2C). The difference in size of the prostate glands cannot be due to depletion as a result of mating, because even though

group-reared animals had unlimited mating opportunities during their development, they were isolated for several weeks after this developmental period. The prostate gland is normally replenished within eight days of copulation (De Boer et al., 1997). Hence, the difference is size cannot be due to having donated sperm, but is caused by developmental differences. The fact that prostate glands get larger when the female function is not initiated, reveals the trade-off between the male and female function in this hermaphrodite during development.

Van Duivenboden (1983) showed that the receipt of semen accelerates the onset of egg laying. Her results may allude to the presence of an allohormone in the semen, which initiates female reproductive processes in the partner (Koene & Ter Maat, 2001, 2002). One candidate substance that could induce the onset of female reproduction is the egg-laying hormone (CDCH), which is present in the semen (Van Minnen et al., 1989). The earlier onset of the partner's female function would increase male reproductive success of the sperm donor, and would therefore be favoured by sexual selection. However, this manipulation may deviate from the optimal resource allocation of the recipient, which may still want to invest in growth. Because the shift in resource allocation towards female reproduction in the sperm recipient is beneficial for the sperm donor but not necessarily for the sperm recipient, this could result in a sexual conflict. The sperm donor could win this conflict by using a sensory trap (Christy, 1995), i.e. manipulating the female function via (a mimic of) a female hormone.

ACKNOWLEDGEMENTS

We thank K. Montagne-Wajer for technical assistance. This research is supported by the Research Council for Earth and Life Sciences (ALW) with financial aid from the Netherlands Organization for Scientific Research (NWO) to JMK.

REFERENCES

Birkhead, T.R., A.P. Møller & W.J. Sutherland (1993). Why do females make it so difficult for males to fertilize their eggs? *J. Theor. Biol.,* 161 : 51-60.

Charnov, E.L. (1982). *Sex Allocation*. Princeton University Press, Princeton N.J.

Christy, J.H. (1995). Mimicry, mate choice, and the sensory trap hypothesis. *Am. Nat.,* 146 :171-181.

De Boer, P.A.C.M., R.F. Jansen, J.M. Koene & A. Ter Maat (1997). Nervous control of male sexual drive in the hermaphroditic snail *Lymnaea stagnalis*. *J. Exp. Biol.,* 200 : 941-951.

De Visser, J.A.G.M., A. Ter Maat & C. Zonneveld (1994). Energy budgets and reproductive allocation in the simultaneous hermaphrodite pond snail, *Lymnaea stagnalis* (L.) : a trade-off between male and female function. *Am. Nat.,* 144 : 861-867.

De Vlieger, T.A., K.S. Kits, A. Ter Maat & J.C. Lodder (1980). Morphology and electrophysiology of the ovulation hormone producing neuro-endocrine cells of the freshwater snail *Lymnaea stagnalis* (L.). *J. Exp. Biol.,* 84 : 239-271.

Geraerts, W.P.M. & J. Joosse (1984). Freshwater snails (Basommatophora). In : *The Mollusca*, Vol.7, *Reproduction.* Tompa, A.S., N.H. Verdonk & J.A.M. van den Biggelaar (Eds.). Academic Press, New York : 141-207.

Geraerts, W.P.M., E. Vreugdenhil, R.H.M. Ebberink & Th.M. Hogenes (1985). Synthesis of multiple peptides from a larger precursor in the neuroendocrine caudo-dorsal cells of *Lymnaea stagnalis*. *Neurosci. Letters,* 56 : 241-246.

Greeff, J.M. & N.K. Michiels (1999). Sperm digestion and reciprocal sperm transfer can drive hermaphrodite sex allocation to equality. *Am. Nat.,* 153 : 421-430.

Hughes, R.N., P.H. Manríquez & J.D.D. Bishop (2002). Female investment is retarded pending reception of allosperm in a hermaphroditic colonial invertebrate. *Proc. Natl. Acad. Sci. USA,* 99 : 14884-14886.

Koene, J.M., R.F. Jansen, A. Ter Maat & R. Chase (2000). A conserved location for the central nervous system control of mating behaviour in gastropod molluscs : Evidence from a terrestrial snail. *J. Exp. Biol.,* 203 : 1071-1080.

Koene, J.M. & A. Ter Maat (2001). “Allohormones” : A class of bioactive substances favoured by sexual selection. *J. Comp. Physiol. A,* 187 : 323-326.

Koene, J.M. & A. Ter Maat (2002). The distinction between pheromones and allohormones. *J. Comp. Physiol. A,* 188 : 163-164.

Pen, I. & F.J. Weissing (1999). Sperm competition and sex allocation in simultaneous hermaphrodites : A new look at Charnov's invariance principle. *Evol. Ecol. Res.,* 1 : 517-525.

Ter Maat, A., F.A. Dijcks & N.P.A. Bos (1986). *In vivo* recording of neuroendocrine cells (caudo-dorsal cells) in the pond snail. *J. Comp. Physiol. A,* 158 : 853-859.

Ter Maat, A., J.C. Lodder & M. Wilbrink (1983). Induction of egg-laying in the pond snail *Lymnaea stagnalis* by environmental stimulation of the release of ovulation hormone from the caudo-dorsal cells. *Int. J. Invert. Reprod.,* 6 : 239-247.

Ter Maat, A., A.W. Pieneman, J.T. Goldschmeding, W.F.E. Smelik & G.P. Ferguson (1989). Spontaneous and induced egg laying behavior of the pond snail, *Lymnaea stagnalis*. *J. Comp. Physiol. A,* 164 : 673-683.

Van der Steen, W.J., N.P. Van den Hoven & J.C. Jager (1969). A method for breeding and studying freshwater snails under continuous water change, with some remarks on growth and reproduction in *Lymnaea stagnalis* (L.). *Neth. J. Zool.,* 19 : 131-139.

Van Duivenboden, Y.A. (1983). Transfer of semen accelerates the onset of egg-laying in female copulants of the hermaphrodite freshwater snail, *Lymnaea stagnalis*. *Int. J. Invert. Reprod.,* 6 : 249-257.

Van Duivenboden, Y.A. & A. Ter Maat (1985). Masculinity and receptivity in the hermaphrodite pond snail, *Lymnaea stagnalis*. *Anim. Behav.,* 33 : 885-891.

Van Minnen, J., R.W. Dirks, E. Vreugdenhil & J. Van Diepen (1989). Expression of the egg-laying hormone genes in peripheral neurons and exocrine cells in the reproductive tract of the mollusc *Lymnaea stagnalis*. *Neuroscience,* 33 : 35-46.

Zonneveld, C. (1993). *Animal energy budgets : a dynamic approach*. Ph.D. Thesis, Vrije Universiteit Amsterdam.

Zonneveld, C. & S.A.L.M. Kooijman (1989). Application of a dynamic energy budget model to *Lymnaea stagnalis* (L.). *Funct. Ecol.,* 3 : 269-278.

Belg. J. Zool., 134 (supplement 1) : 47-50 March 2004

Evolution : the problem solving strategy as the basic unit of adaptation ?

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ABSTRACT. The theory of evolution would gain in acceptance if it were better able to banish the tautologies that continue to dominate some of its formulations. The principles of communication/problem solving might be helpful in this respect. For example, adaptation as "the evolutionary process by which an organism becomes fitted to its environment" could be replaced by : "adaptation concerns the solving of a particular (set of) problem(s) in a given environment/context, making use of preexisting problem solving strategies". 'Survival of the fittest' says nothing more than that the survivors survive. An alternative could be : "If they are not prematurely eliminated by accidental death, the best problem solvers have better chances for being rewarded with a higher level of contentment, and by faster growth and reproductive advantages". Problem solving requires adequate hardware, software, energy as well as the proper motivation.

KEY WORDS : selection, microevolution, macroevolution, megaevolution.

INTRODUCTION

Evolution is usually considered as the development of new types of living organisms from preexisting types by the accumulation of genetic differences over long periods of time (Henderson, 1995). The recent (2002) book of Stephen Jay Gould, the godfather of American evolutionary biologists, entitled "The structure of evolutionary theory", clearly illustrates the enormous emphasis that contemporary evolutionary theory puts on genetic mechanisms. In this 1433 page book, Gould, by almost completely neglecting cultural evolution, the principles of communication and of problem solving, gives the impression that these are non-issues in (his) evolutionary theory. However, evolution can also be considered as the (evidently unconscious, non-directional) invention, application and accumulation, again and again, of novel strategies for solving problems imposed by changing living conditions, whatever their nature. A possible result is the appearance of new phenotypes and eventually new species. These two approaches will involve quite different ways of thinking for unraveling the nature of the driving forces of evolution as well as of the basic unit of adaptation/selection. The first approach almost exclusively focuses on genetics. The second approach takes into account the whole machinery that is required for problem solving, namely communication with its four pillars (Fig. 1) : hardware (the body of organisms/compartments), software (to decode incoming messages), energy and motivation (De Loof, 2002).

SOME BASIC PRINCIPLES OF COMMUNICATION

Although everybody knows that communication is very important for living organisms, its principles are seldom dealt with in common introductory textbooks of general biology. This is mainly owing to the fact that communication is experienced as being so normal and self-evident that it does not deserve an effort to get insight in its basic principles. However, communication is a much more complex activity than one usually realizes. Hence, it may not be superfluous to elaborate briefly on its principles.

Communication can be defined as transfer of information. The basic functional unit in communication is not 'the cell' but the 'communicating compartment'. Such a compartment consists of a sender that releases a message that is invariably written in *coded form*. This message is then transported through what is called a transmission channel (the air, the blood stream, an axon etc.) towards a competent receiver, thus a receiver that has receptors that can somehow sense/bind the coded message. Next the receiver has to decode and amplify the message, making use of preexisting programs for decoding. Finally, to respond to the message, the receiver has to mobilize part of the energy that it had stored beforehand. Very often, feedback mechanisms are activated next. The receiver becomes a sender and the original sender, and perhaps other entities as well, becomes a receiver. There are numerous families/types of messages and they all require specific decoding mechanisms. Decoding information is not possible without a memory system. There are two types of memories, the genetic memory (in DNA, RNA) and the cognitive memory (molecular carrier not yet known), both with their specific sets of rules/dogma. It is seldom realized that any act of communication involves an act of problem solving, namely as to how the receiver should decode the incoming message(s). What we call 'communication activity' is in fact a synonym for 'unconscious problem solving' (the automaton aspect of life). Humans reserve the term 'problem solving' for that very

tiny part of problems that they cannot automatically solve but that require conscious thinking and handling. However, both use exactly the same principles (for details, see De Loof, 2002).

From the viewpoint of possible sources of variability – essential for understanding evolution – there are many ways in which the activity of a communicating compartment can change : the structure of the sender, the transmission channel, the receiver, the storage and use of energy, the (genetic and cognitive) memory systems, the electrical phenomena that are essential to communication, the interactions with other levels of compartmentalization etc. Thus, changes in genes are only part of the causal mechanisms underlying evolutionary change. The evolution of the hardware of living systems is mainly governed by changes in gene structure/activity, according to the rules of the first central dogma (DNA→RNA→Proteins). What is called 'cultural evolution' is in fact evolution 'the software way' and happens primarily according to the rules of a second, as yet only partially defined dogma (for a sketchy outline of this second dogma, see De Loof, 2002), that deals with the processing of cognitive information. Here teaching-learning activities are of primordial importance.

EVOLUTION OF 'LIFE', THUS OF 'PROBLEM SOLVING ACTIVITY' AT DIFFERENT LEVELS OF COMPARTMENTAL ORGANIZATION

Organisms can solve problems, not because they have genes (they evidently need genes), but because they are constructed as communicating compartments. The type of problems a given compartment – or level of compartmental organization – can solve partially depends upon its architecture. A bacterium (= monomembrane compartment) can solve a given set of problems. A eukaryotic cell (= multimembrane compartment) can handle problems related to the interaction among its cell organelles. A segmented animal can handle problems on at least six successive levels of hierarchical organization : the cell organelle, the eukaryotic cell, aggregate, the syncytium, the monoepithelium, and the polyepithelium. In fact, not only metazoan organisms contain in themselves a series of layers of compartmentalization but the whole biosphere is compartmentally organized in layers of interacting communicating/problem solving compartments. My communication-based classification system comprises 16 levels arranged into three major groups (De Loof, 2002). The first group comprises levels of compartmentalization that can occur within one and the same individual. Already eight levels can be discerned, the major ones being the bacterium/ cell organelle, the eukaryotic cell, the aggregate, the syncytium, the monoepithelium, the polyepithelium, the segmented animal, and animals with their tools. The second group comprises compartments that are formed by more than one individual but still belonging to the same species. This group comprises six additional levels : the colony, the heterosexual couple, social compartments, organism-in-organism (by internal budding) compartments, the population/species compartment, and the electrosphere-compartments. The third group lists compartments consisting of more than one individual but now belonging to different species, e.g. host-parasite associations, food chains, and the highest level of all, the whole biosphere or Gaia compartment. All these levels represent key revolutions that are the heart of mega-evolution. Mega-evolution deals with evolution of life in its totality, more specifically with the revolutions underlying the coming into existence of ever higher levels of compartmental organization, all hierarchically organized. Mega-evolution crosses the borders of systematic classification. The basic principles underlying the coming into existence of the successive levels are surprisingly few in number, and simple : internalization of compartments, gluing together of compartments, utilization of tools, sexuality and development, and nutritive and protective deficiencies (De Loof, 2002).

In order to maintain itself, any organism has to solve problems on a continuous basis. When it enters a new environment, an organism will only survive and reproduce if it can instantly solve the problems that changing environmental conditions impose upon its physiology. From this point of view, a comprehensive theory of evolution should best be based upon universally valid cell biological principles that make problem solving – and hence adaptation – possible.

In my opinion, *the* question in evolution is not : "How do new species come into existence?" (= microevolution) as originally suggested by Darwin's "The Origin of species by means of natural selection or the preservation of favoured races in the struggle for life" (1859) but "How does *life* evolve?". Many biologists have as yet intuitively assumed that the known mechanisms of micro- and macroevolution should suffice to explain the evolution of life in its totality. However, the persisting impotence to provide a cell biological model by which 'cultural evolution' can be made an integral part of neo-Darwinism/the synthetic theory suggests that this is not the case. The only way to overcome this problem is to define life first. Contrary to what nearly everybody thinks, this can be done in a rather simple way as outlined in "Communication, Life, Mega-Evolution – Decrypting Life's Nature" (De Loof, 2002). Details cannot be given here. To make a long story short, life is not just a machine as so many biologists tacitly assume (Rosen, 1991), but the *activity* of a special sort of machine. In my opinion, what we call 'life' is simply an umbrella term denoting the total communication/ problem solving activity of hierarchically organized communicating compartments. This approach shifts the emphasis from 'genes make proteins' to 'genes are one of the elements in the whole problem solving machinery'.

IS PROBLEM SOLVING ACTIVITY LINKED TO SOME 'URMOTIVATION'?

As humans we know that engaging in an activity requires energy as well as the proper motivation. Motivation – an anthropomorphic term for lack of better – is different from the 'software' that is needed to solve particular problems. Because we cannot judge whether organisms other than humans make use of motivation to start mobilizing part of their stored energy to solve problems, some people may hesitate to extrapolate motivation

beyond the human species. My view is that, as is also the case for feelings, motivation did not come into existence out of nothing at the moment that *Homo sapiens* became a distinct species. Most likely, we inherited the underlying mechanisms from very distant ancestors. I think that some form of 'urmotivation' must have been in place already right at the moment life started on earth.

Humans link motivation to processes in the brain, in which electrical phenomena play a crucial role. However, such phenomena (changes in transmembrane voltage gradient, transcellular currents) are not restricted to the nervous system, not even to animals but they operate in any cell type, no matter whether this cell is prokaryotic or eukaryotic. In my opinion the role of the electrical dimension (De Loof, 1986) present in all living systems has as yet been undervalued, not to say almost completely neglected, in theories of evolution.

I think that motivation is linked to the feeling of contentment (again an anthropomorphic term for lack of an appropriate alternative) that, when it turns negative into discontentment, informs – through appropriate signaling pathways – organisms/compartments that some action is required to cope with an emerging problem. Gradually biologists start to recognize that senses and feelings are more universal than generally assumed (Ford, 2000). No doubt, the unraveling of the full molecular basis of any feeling will require a lot of additional research : at present, we are only in the early exploration phase.

If the definition of life that I proposed is correct, the consequence is that life can change by alterations in the hardware (mutations), in the software needed to solve problems (mutations and learning processes), in energy or in motivation. Most of the time, all four pillars of life (Figure 1) are simultaneously subject to change.

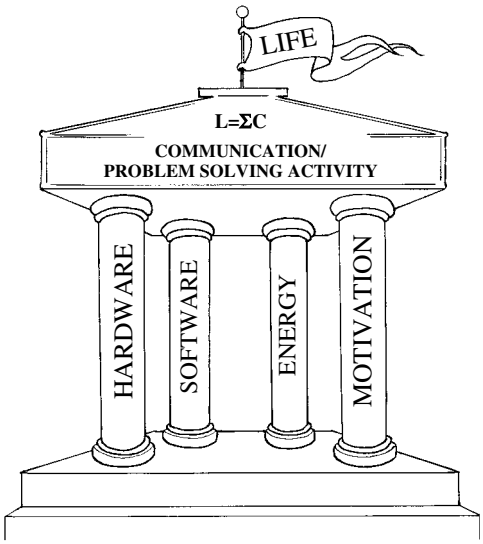

Fig. 1. – The four pillars of "life".

EVOLUTION IS FARSIGHTED BLIND

When approaching evolution from the viewpoint of problem solving, it immediately becomes clear that a given problem can only be solved if the whole machinery is in place at the moment the problem manifests itself. This requires that strategies for problem solving have to be elaborated (long) beforehand (e.g. in gametes), thus in a period when that particular problem does not yet exist. I call this "Evolution is farsighted blind". The more such problem solving strategies are accumulated in organisms/populations/compartments, the more chances their progeny will have to successfully cope with future adverse conditions. Thus, adaptive success is the *result* of the activation at the right moment of preexisting problem solving strategies.

THE PROBLEM SOLVING STRATEGY AS THE BASIC UNIT OF ADAPTATION

Adaptation is the successful solving of a particular problem in a given environment/context, making use of preexisting problem solving strategies. In my view, the basic and universal unit of adaptation is *the problem solving strategy*. As long as the second central dogma is not fully understood, the full description down to the molecular level of such strategies will not be possible.

SELECTION. WHO SURVIVES : THE 'FITTEST' OR THE BEST PROBLEM SOLVERS?

I do not favor the view that there exists something as 'a unit of selection' as put forward by Gould (2002) rather than a unit of adaptation. In my opinion, selection is itself the result of something that happened before in the context of problem solving. The view, held by some researchers, that the gene could be the basic unit of selection cannot be correct because problem solving requires more than just genes. Neither is the view justified that the organism is the universal unit (see Gould, 2002). Indeed, organisms can only solve problems at their specific level of compartmentalization, not at any other higher or lower level. The type of problem that has to be solved is usually linked to a given level of compartmentalization. I think that the two adages, namely 'struggle for life' and 'survival of the fittest' should be replaced by better ones. Indeed, because organisms are not aware of the meaning of death, they cannot 'struggle for life'. Replace this by 'Organisms solve problems or they don't'. 'Survival of the fittest' is a tautology that says that the survivors survive. Replace this by : "If not accidentally eliminated, the best problems solvers have more chances to be rewarded with better growth and possibilities for reproduction."

SUMMARY OF THE ESSENTIALS OF MICROEVOLUTION AS FORMULATED FROM THE VIEWPOINT OF PROBLEM SOLVING

The Darwinian concepts of 'struggle for life' and 'survival of the fittest' as applicable to micro-evolution can perhaps be made less harsh and more all- encompassing when viewed in the following perspective :

– Organisms live in changing environments.
– They have means for sensing changes in gradients of temperature, humidity, salinity etc.

– Organisms are comfort-lovers.
– Their basic feeling is of 'contentment'.
– All sorts of influences can disturb this 'state of contentment'.
– Hence all organisms must solve problems in order to regain their state of contentment and/or improve their quality of life.
– Their basic architecture as communicating compartments with their hardware, software and energy aspects allows endless variability. It generates the possibility for problem solving and it also contains the genetic and cognitive memories and mechanisms for anticipating solutions to future problems and for adaptation.
– The two types of memory are likely to involve two differential central dogmas. The genetic memory obeys the rules of the first central dogma : DNA → RNA→ Protein(s). A second central dogma, governing the cognitive memory, is likely to exist as well. Its nature is as yet only partially determined, but electrical phenomena are surely part of it – and perhaps the actin cytoskeleton as well.
– If they are not prematurely eliminated by accidental death, the best problem solvers (=the fittest) have more chances for being rewarded with a higher level of "contentment", and by faster growth and reproductive advantages.

All levels of compartmental organization are subject to evolutionary changes. The full formulation of this concept (in 20 points) can be found in De Loof (2002).

CONCLUSION

Darwinism is a splendid theory but it deserves a better formulation, preferably in contemporary language, than is presently the case. The vocabulary of informatics/computer sciences is appropriate in this respect. An (partial) attempt in this direction has been undertaken by De Loof (2002) and is given in abbreviated form here.

REFERENCES

Darwin, C. (1859). *The origin of species by means of natural selection or the preservation of favoured races in the struggle for life*. First published by John Murray.

De Loof, A. (1986). The electrical dimension of cells : the cell as a miniature electrophoresis chamber. *Int. Rev. Cytol.*, 104 : 251 352

De Loof, A. (2002). *Communication, Life, Mega-evolution. Decrypting Life's Nature*. Leuven University Press, Leuven.

Ford, B.J. (2000). *The secret language of Life. How animals and plants feel and communicate*. Fromm International Publishers, New York.

Gould, S.J. (2002) *The structure of evolutionary theory.* The Belknap Press of Harvard University, Cambridge, Massachusetts.

Henderson (1995). *Henderson's Dictionary of Biological Terms* (11th edition). Longman Scientific and Technical, Harlow, England.

Rosen, R. (1991) *Life itself. A comprehensive inquiry into the nature, origin, and fabrication of life*. Columbia University Press.

Belg. J. Zool., 134 (supplement 1) : 51-54 March 2004

Cuticular lobes in the *Tetranychus urticae* complex (Acari : Tetranychidae) : a reliable taxonomic character?

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ABSTRACT. Taxonomic work on spider mites has mostly been based on morphological characters. The shape of lobes on dorsal integumentary ridges of summer females was used in the past as an additional morphological character to discriminate between the green and the red forms of the two-spotted spider mite *Tetranychus urticae*. However, we showed that lobes were not always present or were not completely formed in some females, presumably because of micro-environmental conditions. No clear-cut differences were put forward between green and red forms concerning that ridge structure. This underlines the care needed when this criterion is used. The possible role of lobes in adaptation to water balance is discussed.

KEY WORDS : Acari; spider mites, *Tetranychus urticae*; cuticular lobes, taxonomy.

INTRODUCTION

Descriptions of species of Tetranychidae (Acari) are mostly based on morphological characters. However, in the *Tetranychus* genus, some species are very difficult to distinguish as the identification is based on small differences in characters that express a range of variations. For instance, KOCH (1836) gave the first denomination *Tetranychus urticae* in his description in 1836. The mite described by KOCH was collected in Germany on *Urtica dioica*. Nevertheless, two forms are recorded, a green and a carmine or red form. Both forms are morphologically very similar and have a worldwide distribution. However, the green form is generally found in cold and temperate climates, while the red form occurs over much of the warmer temperate zone and subtropics (DUPONT, 1979). In the literature, important confusion exists due to the unclear form or species status resulting in the existence of numerous synonyms (VAN IMPE, 1991). BOUDREAUX (1956) raised the green and red forms to species status on the basis of breeding experiments and morphological characters such as the shape of cuticular lobes of adult summer females. BOUDREAUX indicated that red summer females (renamed by him *Tetranychus cinnabarinus*) bear narrow pointed cuticular lobes, while green summer females (*Tetranychus urticae)* bear broader and more rounded cuticular lobes. Recognition of these species depends thus almost entirely upon observations made on the cuticle, since colour was not considered as a reliable character in the revision of the spider mite family Tetranychidae by PRITCHARD & BAKER (1955). The value of the shape of dorsal lobes in females as a morphological character is subject to criticism. Indeed, the variation is difficult to interpret because of overlapping characters in both forms (VAN DE BUND & HELLE, 1960); lobes must be viewed in the same position (DOSSE & BOUDREAUX, 1962); dorsal strial lobe densities change with temperature and humidity (MOLLET & SEVACHERIAN, 1984); green mites were even identified as *Tetranychus cinnabarinus* when shape of lobes and number of setae on tibia I were used as morphological characters (ZHANG & JACOBSON, 2000). However, lobes were also used to distinguish between strains of two-spotted spider mites (HANCE *et al.,* 1998), using a biometrical approach.

Little information is available on the relation between integumental structures and their physiological functions for *Tetranychus urticae*. The definition of the lobes and their description are vague : the cuticle of the spider mite is supplied with numerous parallel ridges, and lobes are the result of the presence of incisions in these cuticular ridges (BOUDREAUX & DOSSE, 1963). The lack of information on cuticular structures provokes contradictory observations. Indeed, according to Boudreaux, males do not bear lobes on the cuticular folds (BOUDREAUX, 1956). However, in the description of cuticle structures, MOTHES-WAGNER & SEITZ (1984) indicated lobes on the male cuticle. The lack of information about nymphal stages and males is linked to the higher interest of taxonomical characters of females.

In this paper, we examine if the shape of lobes on the mediodorsal hysterosoma (between the third and fourth pair of dorsocentral hysterosomal setae) represents a useful tool to separate summer females within and between colour forms.

MATERIAL AND METHODS

Origins of mites and culture

Mites were collected on several nettle plants (*Urtica dioica*) in Southern France and in Belgium. Two samples of *T. urticae* were collected in July 2000 in France : the first sample came from Anduze (44˚03' N; 3˚59' E) and was composed of red mites, while the second sample

came from the vicinity of Montpellier in Lattes (43˚36'N; 3˚53'E) and was composed of green mites. One sample was collected in August 2000 in Belgium, in Rixensart (50˚42'N; 4˚32'E) and was composed of green mites.

Mites were reared for about one month in a conditioned temperature-humidity room held at 20˚C ± 3˚C, relative humidity 80%, and photoperiod 16/8 L/D. As these conditions were rather unfavourable (some females showed a winter orange colour, did not feed and did not lay eggs), three weeks before preparation of mites for observations, conditions were changed to : temperature 24˚C ± 0.5˚C, relative humidity 50%, photoperiod unchanged. This caused all females to attain summer colour, to start feeding and laying eggs. Then, for each population, ovipositing females were placed on isolated detached bean leaves in boxes filled with moist cotton wool. After 24 hours, all adult females were removed and the eggs were left until their development into adults. These adults were the observed mites.

Scanning Electron microscopy

Sister adult females were taken between three and four days after emergence. Individual females were taken with a small brush and glued onto metal plates. For scanning electron microscopy (SEM Philips XL20), specimens were flash frozen (-212° C) in liquid nitrogen under vacuum for cryo-SEM (Oxford CT1500 cryo-system), transferred to the preparation chamber, and then to the SEM chamber where, when the cuticle was moist, the frozen samples were sublimated (-80° C) to remove ice particles on the summits of the lobes. Specimens were viewed under 2--5 KV at 170 to 190° C.

Observations were made on the standard mediodorsal hysterosoma region. The longitudinal striae between the third and fourth pair of dorsocentral setae form a diamond-shaped figure between these setae (Tuttle & Baker, 1968). Observations were made in this area, near to the third dorsocentral hysterosomal setae. Eight red females from Anduze, five green females from Lattes and 12 green females from Rixensart were observed.

RESULTS

Pinpointing of the diamond-shaped figure on the hysterosoma is easy and is indicated in Fig. 1. Each female bears a different shape of this diamond-shaped figure. The size of the figure is quite similar but the outline varies particularly at the meeting points of the sides. This diamond-shaped figure is only present in adult females. In this area, the lobe size of our females was very variable. Indeed, some green and red individuals were characterised by typical lobes while others did not possess lobes or only less-developed lobes. The sampling location, mite colour and observation results are shown in Table 1. Figures 2 and 3 show the typical lobes for the red and green females. The lobes of green females were mostly semi-circular to oblong and wide, while sometimes triangular lobes were observed. The lobes of red females were mostly triangular to semi-circular and taller than on the green form; the lobes were generally more separated at their base. Figures 4 and 5 show less-developed and smooth lobes. In this case, it is impossible to distinguish between the green and the red forms. The variability in lobe development was high although the mites were related and reared in the same environmental conditions, and all observed females showed the colour of summer females : green or red according to the population.

TABLE 1

Presence and absence of lobes for the observed mites.

sampling location	Mite colour	Number of observed females	Number of females with typical lobes	Number of females without obes or with less developed lobes
France (Anduze)	Red	8	6	2
France (Lattes)	Green	5	3	2
Belgium (Rixensart)	Green	12	12	0

DISCUSSION

As shown in many studies (Van de Bund & Helle, 1960; Dosse & Boudreaux, 1962; Boudreaux & Dosse, 1963; Hatzinikolis, 1970), both green and red forms of the two-spotted spider mite *Tetranychus urticae* exhibit typical lobes as described by Boudreaux (1956). However, shape variability is found and some mites bear less-developed or smooth lobes. This variability weakens the reliability of this character for distinguishing between the forms and for comparing mites within a colour form. We found that separation between forms is not as clear as has been reported in the past (Boudreaux, 1956; Dosse & Boudreaux, 1962). Nevertheless, individual variability has already been pointed out (Van de Bund & Helle, 1960). Dosse & Boudreaux (1962) had criticized this last study by reporting that mites were improperly observed, however, at the same time they explained the variability by the occurrence of hybrid populations. Hybrids were also observed by Dupont (1979). Variability in dorsal strial lobe densities was also noticed, and linked to temperature and humidity (Mollet & Sevacherian, 1984).

Twenty-five females were analysed in this study. However, variability of lobe development was unexpectedly high, even for females coming from the same location. Indeed, if females bear less-developed or smooth lobes, it is impossible to relate the mite colour to lobe observations. Variability interpretation is difficult because this variability was noted while observed females were related and reared, after field collection, under the same environmental conditions. The differences we observed did not depend upon age as females were almost of the same age. A part of the variability recorded in the literature could be the result of individual preparation and, for instance, the way of mounting individuals for microscopy. In our case this bias is drastically reduced, as individuals were not dried to a critical point but handled directly in the microscope using liquid nitrogen. Critical point drying needs, indeed, successive baths in acetone or ethanol solutions that could dissolve or damage some structures. Our method allowed the preservation of the whole cuticle structure.

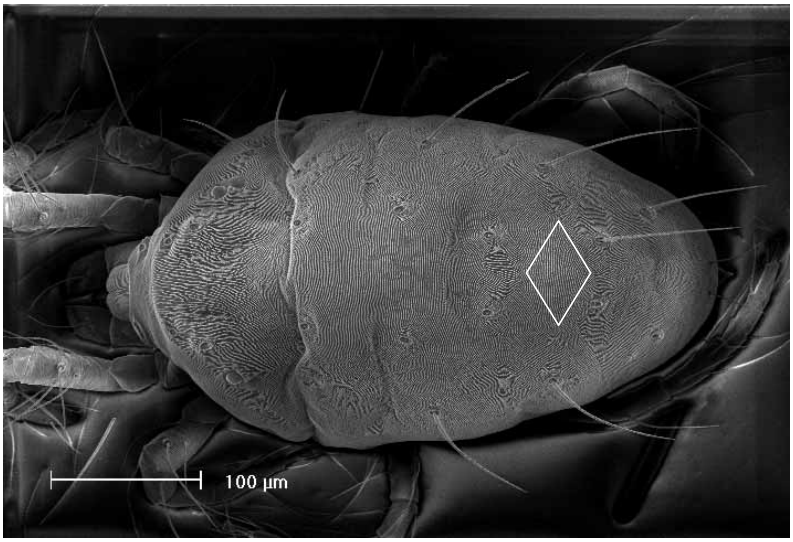
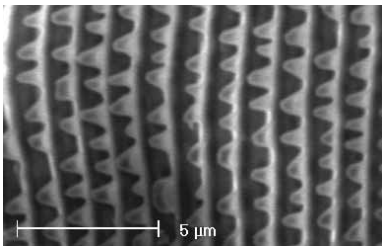


Fig.1



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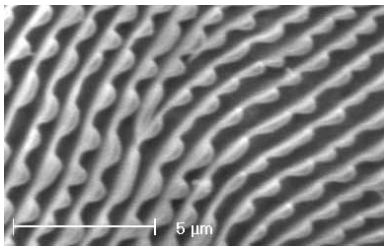

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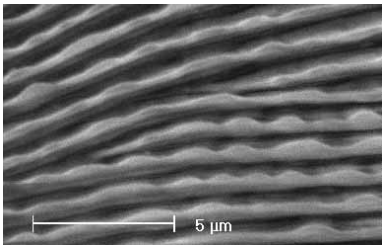

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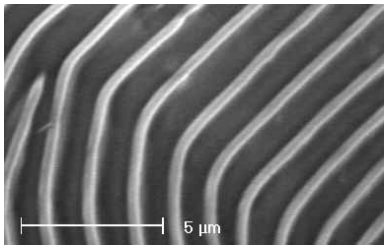

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Fig. 1. – A female *T. urticae*. The white diamond indicates the observed region of the dorsal cuticle.
Fig. 2. – Typical triangular or semicircular lobes of feeding red females. This female was sampled in Anduze (France).
Fig. 3. – Typical semi-oblong lobes of feeding green females. This female was collected in Lattes (France).
Fig. 4. – Less-developed lobes of a feeding green female. This female was collected in Lattes (France).
Fig. 5. – Smooth lobes of a feeding red female. This female was collected in Anduze (France).

Concerning the variability we observed in lobe development, an explanation could be found in the period of observation and in environmental conditions. Indeed, mites were firstly maintained in the laboratory in less than favourable conditions, during which time some females presented diapausing symptoms (orange, non-feeding and non-ovipositing). Subsequent modifications to the temperature and humidity conditions induced formation of the next generations of active individuals (green or red feeding mites according to the population). The observed feeding mites should show lobes, but it is possible that they were still affected by environmental conditions and by the environmental conditions experienced by their parents. Moreover, despite these controlled conditions, we cannot exclude completely the existence of small variations resulting from position of mites within the boxes. According to MOLLET & SEVACHERIAN (1984), humidity and temperature affect the dorsal strial lobe densities. It could partly explain the absence of lobes in some active females, but more experiments and information are needed to enable clear conclusions to be drawn. Our results suggest that care must be taken in the use of this morphological character in taxonomical work.

Concerning the role of lobes, BOUDREAUX (1958) hypothesised that changes in cuticular morphology may be due to differences between summer and diapause forms. Indeed, diapausing females lack lobes over much or all of their body. In summer active females, lobes may serve as an evaporative structure; the absence of that structure in diapausing (non-feeding) females would help them to conserve water. MILLER suggested another possible role by a personal communication to HENNEBERRY et al. (1965); the cuticular extensions could aid mite camouflage by giving the cuticle a relatively lustreless appearance.

DE BOER (1985) assumed some adaptive significance of the pigmentation associated with climate. Indeed, red and green forms display a worldwide distribution, but the green one is mostly present in cold and temperate climates while the red form occurs over much of the warmer temperate zone and subtropics (DUPONT, 1979). If in spite of variability, the shape of lobes differs according to colour forms, this could also indicate that shape corresponds to an adaptation to climate. The variability noticed in our study could perhaps be induced by very limited changes in abiotic conditions at the individual level. Moreover, observations were made after several generations from the collected mites; the variability could reflect a plasticity of the character.

Usefulness of shape of lobes as a taxonomical character is always subject to discussion, and at present it does not help us distinguish between mites as long as its precise role remains unknown. Recently, green mites were identified as *T. cinnabarinus* using shape of lobes and other morphological characters (ZHANG & JACOBSON, 2000). Up to now, green mites have never been reported as *T. cinnabarinus.* This shows the complexity in mite identity. Moreover, biological information such as breeding experiments sometimes show reproductive compatibility, partial compatibility or reproductive incompatibility (HELLE & PIETERSE, 1965).

In the future, studies on the physiological processes of the cuticle and its structure at each stage could help us understand the roles and the variability during the mite life according to environmental conditions. Moreover, molecular analyses may help to define more precisely the status of the different populations and to determine if the recorded variability could be due to a phenotypic plasticity or a genetic variability.

ACKNOWLEDGEMENTS

This work was supported by “Fonds pour la Formation à la Recherche dans l’Industrie et dans l’Agriculture (FRIA)” of Belgium. The authors thank Cony Decock (BCCM/MUCL) for assistance with microscope observations and with photography. This publication is publication BRC 020 of the Research Center on Biodiversity, UCL.

REFERENCES

BOUDREAUX, H.B. (1956). Revision of the two-spotted spider mite (Acarina, Tetranychidae) Complex, *Tetranychus telarius* (Linnaeus). *Ann. Entomol. Soc. America*, 49 : 43-49.

BOUDREAUX, H.B. (1958). The effect of relative humidity on egg-laying, hatching, and survival in various spider mites. *J. Insect Physiol*., 2 : 65-72.

BOUDREAUX, H.B. & G. DOSSE (1963). The usefulness of new taxonomic characters in females of the genus *Tetranychus* Dufour (Acari : Tetranychidae). *Acarologia*, 1 : 13-33.

DE BOER, R. (1985). Reproductive Barriers. In : *World Crop Pests. Spider Mites. Their Biology, Natural Enemies and Control. Volume1A.* HELLE, W. & M.W. SABELIS (Ed.). Elsevier, Amsterdam, Oxford, New York and Tokyo : 196.

DOSSE, G. & H.B. BOUDREAUX (1962). Some problems of spider mite taxonomy involving genetics and morphology. *Advances in Acarology,* 1 : 343-349.

DUPONT, L.M. (1979). On gene flow between *Tetranychus urticae* Koch, 1836 and *Tetranychus cinnabarinus* (Boisduval) Boudreaux, 1956 (Acari : Tetranychidae) : synonymy between the two species. *Entomol. Exp. Appl*., 25 : 297-303.

HANCE, TH., NEUBERG, P. & CH. NOÈL-LASTELLE (1998). The use of fecundity, lobe bimetry and the RAPD-PCR technique in order to compare strains of *Tetranychus sp. Exp. Appl. Acarol*., 22 : 649-666.

HATZINIKOLIS, E.N. (1970). Contribution à l'étude de l'espèce *Tetranychus telarius* (linnaeus, 1758) (complex) (Acarina : Tetranychidae). *Annls Inst. phytopath. Benaki N.S*., 9 : 207-218.

HELLE, W. & A.H. PIETERSE (1965). Genetic affinities between adjacent populations of spider mites. *Entomol. Exp. Appl.,* 8 : 305-308.

HENNEBERRY, T.J., J.R. ADAMS & G.E. CANTWELL (1965). Fine structure of the Integument of the Two-Spotted Spider Mite, *Tetranychus telarius* (Acarina : Tetranychidae*). Ann. Entomol. Soc. America,* 58 : 532-535.

KOCH, C.L. (1836). *Tetranychus urticae. Deutsche Crustacea, Myriapoda*, 1 : 10.

MOLLET, J.A. & V. SEVACHERIAN (1984). Effect of temperature and humidity on dorsal strial lobe densities in *Tetranychus* (Acari : Tetranychidae). *Internat. J. Acarol.,* 10 : 159-161.

MOTHES-WAGNER, U. & K.A. SEITZ (1984). Fine structure of the cuticle and structural changes occurring during moulting in the mite *Tetranychus urticae* I. Fine structure of the cuticle. *Acarologia,* 25(3) : 253-258.

PRITCHARD, A.E. & E.W. BAKER (1955). *A revision of the spider mite family Tetranychidae.* Memoirs Series volume 2. Pacific Coast Entomol. Soc., San Francisco.

TUTTLE, D.M. & E.W. BAKER (1968). *Spider mites of southwestern United States and a revision of the family Tetranychidae.* University of Arizona Press, Tucson, Arizona.

VAN DE BUND, C.F. & W. HELLE (1960). Investigations on the *Tetranychus urticae* complex in Northwest Europe (Acari : Tetranychidae*). Entomol. Exp. Appl.,* 3 : 142-156.

VAN IMPE, G. (1991). De la difficulté de désigner correctement un organisme vivant. Le cas du Tétranyque tisserand, *Tetranychus urticae* Koch, 1836 (Acari : Tetranychidae). *Bull. Annls. Soc. r. Belg. Ent*., 127 : 71-76.

ZHANG, Z-Q & R.J. JACOBSON (2000). Using adult female morphological characters for differentiating *Tetranychus urticae* complex (Acari : Tetranychidae) from greenhouse tomato crops in UK. *Syst. & Appl. Acarology*, 5 : 69-76.

Belg. J. Zool., 134 (supplement 1) : 55-59 March 2004

Age-dependent morphology and ultrastructure of the thoracic labial gland of *Apis mellifera carnica* Pollm. workers (Hymenoptera, Apidae)

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ABSTRACT. Exocrine glands are well developed in social Hymenoptera and play an important role in the social life of these insects. We studied the thoracic labial gland of *Apis mellifera carnica* Pollm. in relation to age. We observed two cell types in the secretory tubes of the thoracic salivary gland : parietal and secretory cells. In the secretory cells, two types of secretory vesicles occur. Abundant mitochondria, vesicles and apical microvilli indicate that parietal cells also have a secretory function. The size of the subcuticular space increases with age, probably by filling with secretion. We found differences in the numbers of secretory vesicles between summer and winter bees. Probably, these vesicles accumulate in winter bees, and glands do not discharge their secretion until spring.

KEY WORDS : honeybees, exocrine glands, thoracic labial gland, morphology, ultrastructure.

INTRODUCTION

A very striking characteristic of social insects is the extent and development of the exocrine system (Billen & Morgan, 1998). These glands play an important role in the social life of these insects, e.g. maintaining task regulation, which is mainly based on age in worker honeybees. Age-dependent changes in exocrine glands can be expected, because of changing functions. Preliminary studies described these morphological changes in e.g. the wax gland (Cruz Landim, 1963) and the hypopharyngeal gland (Cruz Landim & Hadek, 1969), but only for functional task groups.

Another exocrine gland is the labial or salivary gland that appears in all Hymenoptera and is situated in the ventral part of the thorax, with ducts fusing in the head and opening near the labium (Snodgrass, 1956). The thoracic part of the labial gland of the honeybee belongs to the tubular type (Cruz-Landim, 1973). The secretory cells are of the epithelial type (Noirot & Quennedey, 1974; Billen, 1991). There is also a pair of postcerebral labial glands in the head of the honeybee, which are unique for the Apidae (Snodgrass, 1956). The thoracic labial gland in the honeybee is well described by Schönitzer & Seifert (1990), but not with respect to age.

This paper forms part of a general study that examines the age-dependent morphological and ultrastructural characteristics of the exocrine glands in the honeybee, of which we here focus on the thoracic labial gland.

MATERIAL AND METHODS

Workers of *Apis mellifera carnica* Pollm. were studied. The queen was placed under arrest on two combs to get a concentration of brood, each week on different combs for three weeks. We let workers emerge once a week in a breeding box, after which we marked the callow bees with different colours to know their age. Winter bees were taken on the combs, their age is not exactly known, but was at least two months. Three workers of each age were dissected in Ringer solution (Jolly), fixed in 2% glutaraldehyde buffered with 0.05 M sodium cacodylate. One percent osmium tetroxide was used for postfixation and was followed by dehydration in acetone and embedding in Araldite.

Semi-thin sections (1 µm) for light microscopy were made with a Reichert OmU2 microtome and stained with methylene blue and thionine. Thin sections (70 nm) for electron microscopy were made with a Reichert Ultracut E microtome and double-stained with uranyl acetate and lead citrate. They were examined with a Zeiss EM900 electron microscope.

Samples for scanning microscopy were dehydrated in formaldehyde dimethyl acetal, after which the glands were critical point dried. They were coated with gold and viewed with a Philips SEM 515 microscope.

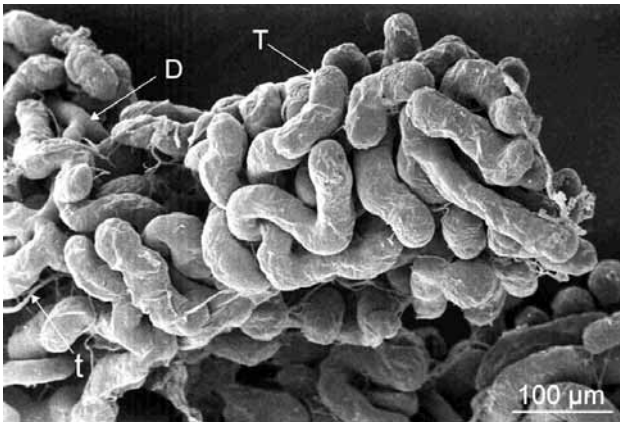

Fig. 1. – Scanning micrograph of the thoracic labial gland in a *A. m. carnica* worker. D : duct; T : secretory tube; t : trachea.

RESULTS

The thoracic labial gland of the honeybee is situated in the ventral anterior part of the thorax. The gland is paired, with two units in each part : a big median and internal lobe and a smaller external lobe. Each lobe consists of a dense cluster of secretory tubes and channels that are sometimes branched (Fig. 1). In this mass we distinguished secretory tubes and collecting tubes with a gradual change between them. Cross sections of secretory tubes show three to five epithelial cells surrounding a central lumen, which is lined with a cuticle with taenidiae (Fig. 2). The region between the gland epithelium and the cuticle is called the subcuticular space (SCHÖNITZER & SEIFERT, 1990). The apical part of the epithelium is very irregular. The cell membrane displays microvilli, which create a surface enlargement to enable a higher secretory capacity. A subcuticular space is also seen between cells. The epithelium contains two types of cells : secretory and parietal cells (SCHÖNITZER & SEIFERT, 1990). The parietal cells are only seen at the periphery (Fig. 3F). They are smaller and more elongated than secretory cells, and have clear vesicles and a high number of mitochondria. The apical cell membranes of the parietal cells appear as a microvillar border. The cytoplasm is not as electron-dense as in the secretory cells. We observed conspicuous basal invaginations in the gland epithelium.

The ducts are clearly distinguishable from the secretory tubes, the cuticle also has taenidiae and is surrounded by epithelial cells, but there is no subcuticular space. These cells have large basal invaginations and apical microvilli with electron-dense zones, which appear to be hemidesmosomes. The basal invaginations become bigger when workers get older. The cytoskeleton is well-developed and microtubules are clearly visible. There is an abundance of mitochondria in the cytoplasm.

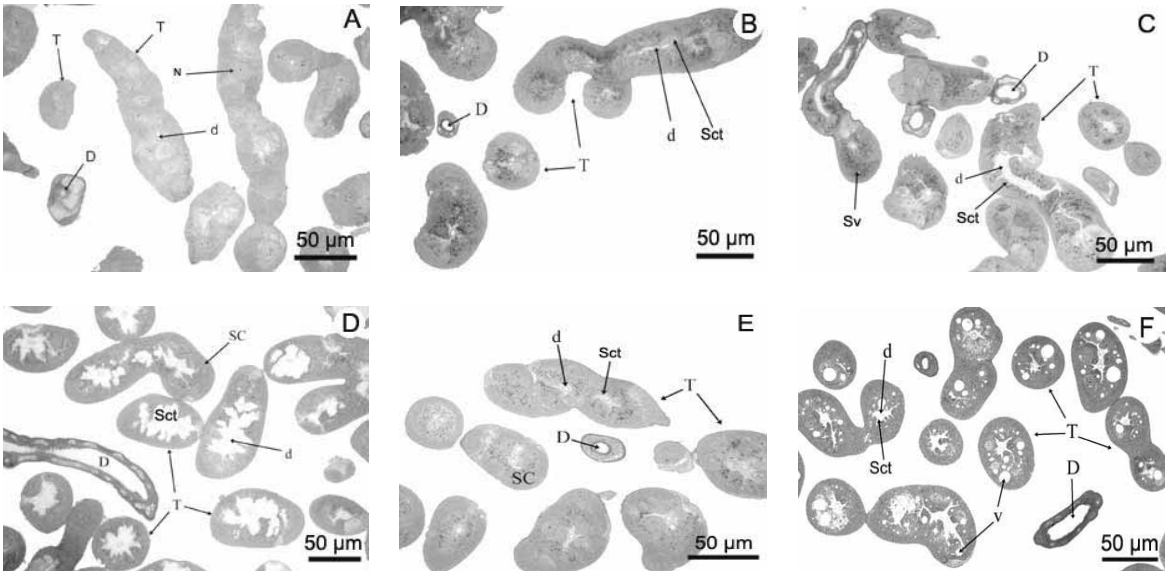

Fig. 2. – Semithin light micrographs of the thoracic labial gland in *A. m. carnica* workers. (A) newly emerged; (B) 3 days old; (C) 9 days old; (D) 15 days old; (E) 24 days old; (F) winter bee. D : duct; d : central duct; N : nucleus; SC : secretory cell; Sct : subcuticular space; Sv : secretory vesicle; T : secretory tube; V : vesicle.

Age-dependent observations

In newly emerged worker bees, we did not see secretory vesicles in the cytoplasm nor secretion in the ducts. The subcuticular space is very small. The nucleus contains several nucleoli. The cytoplasm of the gland cell contains an abundance of mitochondria, mainly between the basal invaginations. Mitochondria are also seen in the apical cytoplasm. Tubular granular endoplasmic reticulum (RER) and free ribosomes are mainly found in the apical region, as well as a well-developed Golgi-apparatus (Figs. 2A – 3A).

At an age of three days we observed secretory vesicles in the apical part of the cytoplasm. The subcuticular space has not grown. Again we see mitochondria at the basal part of the cytoplasm, but larger in size (approx. 0.6 µm). There are electron-dense vesicles visible apically in the cytoplasm. No vesicles are seen in the basal part of the cell. The RER is mainly vesicular and is observed throughout the cytoplasm. A thick (approx. 0.25 µm) basement membrane borders the gland (Figs. 2B-3B).

At an age of six days, the apical side of the gland epithelium is more irregular and the subcuticular space bigger, probably because of accumulation of secretion within it.

At an age of nine days, secretory vesicles are not only seen in the apical part but throughout the cytoplasm, with an increasing density towards the apical side of the gland cell. Two types of gland cells can be distinguished according to the electron density of their cytoplasm. In both types we observed approximately the same number of secretory vesicles in the apical part. Clear vesicles are also seen in both types of gland cells (Figs. 2C-3C). At an age of 12 days the RER is vesicular. The mitochondria on the basal side are directed in a basal-apical direction. Large and clear vesicles are grouped.

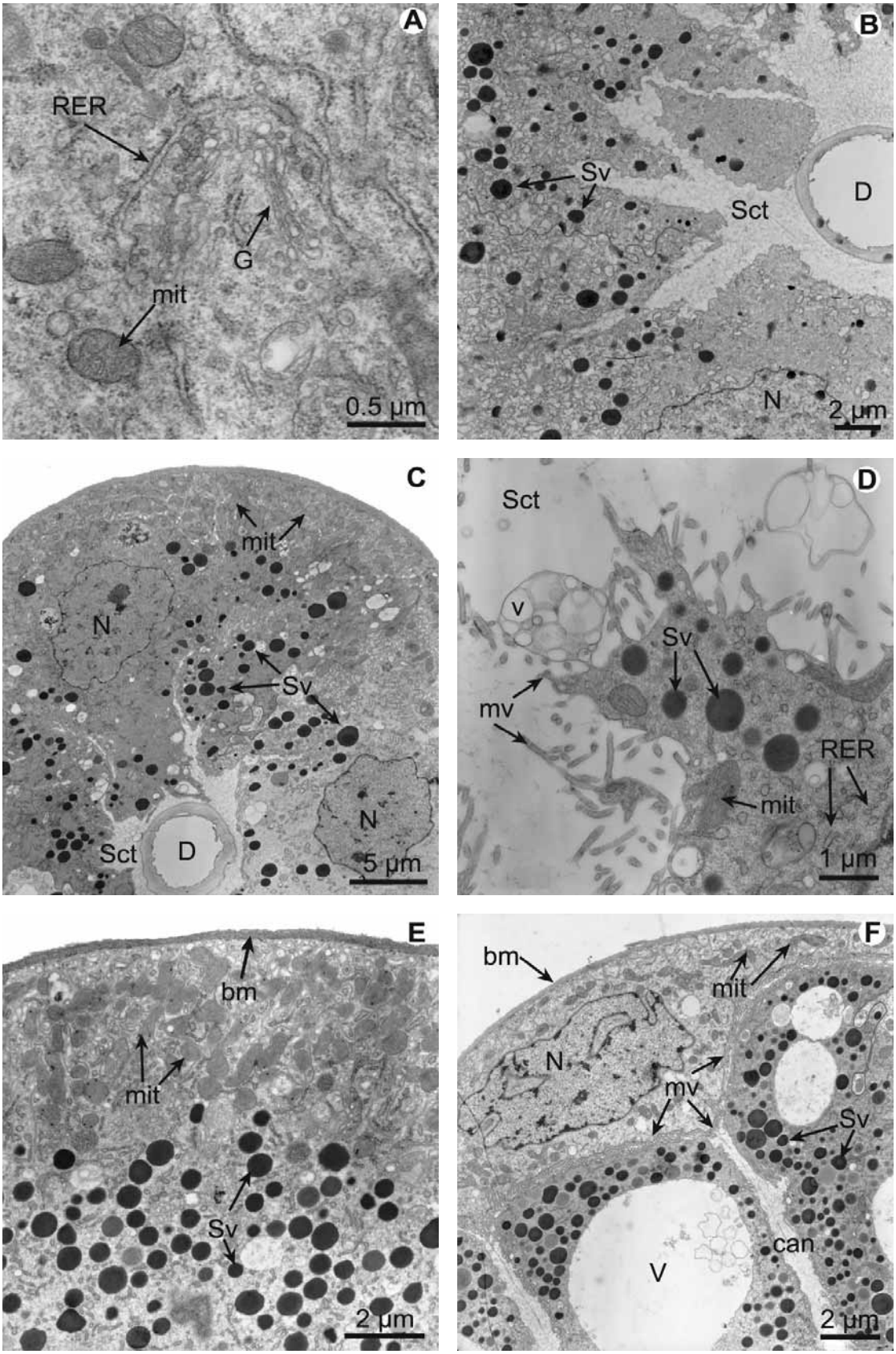

Fig. 3. – Transmission electron micrographs of the thoracic labial gland in *A. m. carnica* workers. (A) newly emerged, detail of cytoplasm; (B) 3 days old, apical side of epithelium; (C) 9 days old, 2 types of secretory cells; (D) 15 days old, apical side of epithelium; (E) 33 days old, basal side of epithelium; (F) winter bee, parietal cell and basal side of epithelium. bm : basement membrane; can : canalicular system; D : duct; G : Golgi apparatus; mit : mitochondria; mv : microvilli; N : nucleus; RER : granular endoplasmic reticulum; Sct : subcuticular space; Sv : secretory vesicle; V : vesicle.

At an age of 15 days, a decrease is seen in the thickness of the gland epithelium. A consequence is an increasing subcuticular space, probably filled with secretion. The canalicular system between the cells is now part of the subcuticular system. The microvilli on the apical side of the gland epithelium are well-developed. In the subcuticular space there are groups of vesicles near the microvilli of the apical part of the gland cell. These probably originate from the cytoplasm. Mitochondria are more electron-dense than before and have a changed internal structure; the cristae are no longer parallel (Figs. 2D-3D). At an age of 18 days the subcuticular space has become smaller. The RER in the cytoplasm of the secretory cells is mainly vesicular. At an age of 21 days the RER becomes more reticular again and at an age of 24 days the RER is tubular. We observed fewer secretion vesicles. No change occurs after this age (Figs. 2E-3E).

In winter bees we saw electron-dense secretory vesicles in the apical part of the secretory cells, as in summer bees. We observed clear spherical inclusions of a different size, scattered over the cytoplasm. Sometimes these inclusions can occupy half of the gland cell. Differences occur between cells in their electron density. The nuclei have a spherical form (Figs. 2F-3F).

No changes were observed in diameter of the secretory tubes or in thickness of the epithelium (Figs. 4-5).

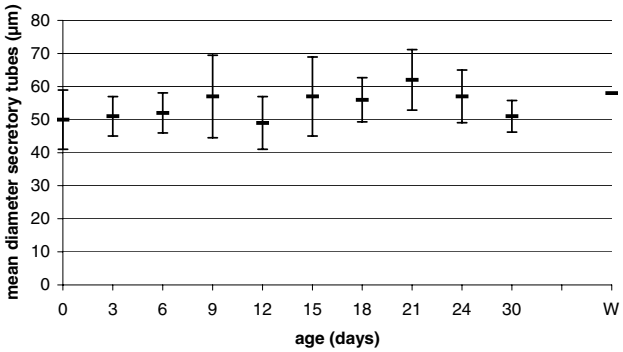

Fig. 4. – Mean diameter of secretory tubes of thoracic labial gland with respect to age (n=3). W : winter bees.

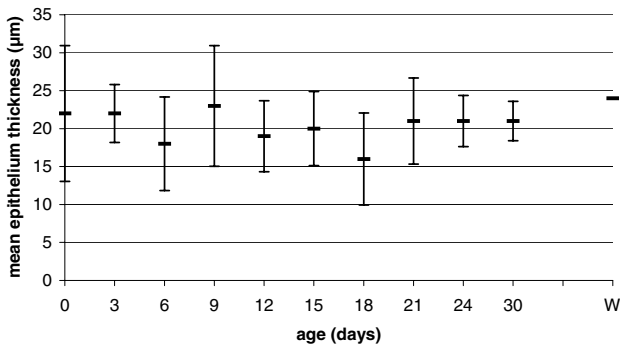

Fig. 5. – Mean epithelium thickness of thoracic labial gland with respect to age (n=3). W : winter bees.

DISCUSSION

The cytoplasm of the duct epithelium contains numerous mitochondria, probably because of an active transport function. Changes in the size of the basal invaginations with age are probably linked to this transepithelial transport. In the secretory tubes, the parietal cells contain many mitochondria, which show great activity. The fact that these cells contain many vesicles and apical microvilli, which are linked with the canalicular system, suggests a secretory function. It may also be that they have a special function regulating the concentration of ions in the saliva, or that secretory cells and parietal cells produce different components of the secretion (Schönitzer & Seifert, 1990).

In the secretory cells we see little variation in the amount of RER, mitochondria and secretory vesicles with respect to age, but in correspondence with the observations of Schönitzer & Seifert (1990), we see a different electron density in the cytoplasm of the secretory cells in the same section. Probably the secretory cycle is not synchronous in all cells. Two types of secretory vesicles with different electron density are found. The production of these two types is independent of age and physiological condition of workers. This corresponds with functions of the gland that are needed over the whole life span, such as production of metabolic enzymes. The secretion is a proteinaceous solution and has invertase activity (Cruz-Landim, 1973). This enzyme catalyses the conversion of nectar to honey. Also other enzymes are found in the secretion, e.g. acid phosphatase, naphtol-AS-BI-phosphohydrolase catalysing protein synthesis, leucine arylamidase for hydrolysis of proteins, α-glucosidase for conversion of nectar into honey and β-galactosidase in younger bees for energy supply (Costa & Cruz-Landim, 2001). The secretion also serves as a wax softener, and is also used for dissolving food (Schönitzer & Seifert, 1990). The presence of RER suggests the secretion is a protein-rich fluid. Only the size of the subcuticular space changes with age, possibly because of filling with secretion. It seems that it has a reservoir-like function. This corresponds with the studies of Reimann (1953). Other studies also suggest that the size of the tubes is variable according to the age and secretory cycle (Costa & Cruz-Landim, 2001), with a peak period between eight and 17 days. This corresponds with certain tasks : cleaning the queen, receiving nectar, pollen storing and nursing. Our quantitative estimates do not show any visible trend (Figs. 4, 5). The subcuticular space is biggest at 15 days. It is possible that at this age there is the largest amount of secretion, as we then see grouped clear vesicles near the microvilli in the subcuticular space, but no electron-dense secretion. The secretion is discharged via the medial duct into the secretory tube. This duct is surrounded with a porous cuticle on which taenidiae-like reinforcements are found (Schönitzer & Seifert, 1990).

In winter bees we see many electron-dense vesicles. These are also apical as in summer bees. We could also observe large clear vesicles with internal lamellar structures. Probably this is an accumulation of the clear secretory vesicles we see in summer bees. The gland in winter bees produces secretory vesicles, but probably does not release the secretion. By such accumulation, this gland is loaded and when circumstances change in spring, they can directly discharge secretion.

We conclude that the production and function of secretion of this gland probably does not change in the life of a honeybee worker, there is only an accumulation of secretion. However, different functions are not excluded and further investigation is required to relate this morphology to function and task regulation.

ACKNOWLEDGEMENTS

We greatly acknowledge the help of K. Collart and J. Cillis in tissue preparation for microscopy.

REFERENCES

Billen, J. (1991). Ultrastructural organisation of the exocrine glands in ants. *Ethol. Ecol. Evol., Special Issue,* 1 : 67-73.

Billen, J. & E.D. Morgan (1998). Pheromone communication in social insects : sources and secretions. In : *Pheromone Communication in Social Insects Ants, Wasps, Bees and Termites.* Vander Meer R.K., M.D. Breed, K.E. Espelie & M.L. Winston (Eds.), Westview Press : 3-33.

Costa, R.A.C. & C. da Cruz Landim (2001). Enzymes present in the thoracic gland extracts from workers and males of *Apis mellifera* (Hymenoptera : Apidae). *Sociobiology,* 37 : 563-569.

Cruz Landim, C. da (1963). Evaluation of the wax and scent glands in the Apinae (Hymenoptera, Apidae). *J. N.Y. Entomol. Soc.*, 21 : 2-13.

Cruz Landim, C. da (1973). Tipos de glândulas salivares do tórax presentes em abelhas (Hymenoptera, Apoidea). *Studia Entomol.,* 16 : 209-232.

Cruz Landim, C. da & R. Hadek (1969). Ultrastructure of *Apis mellifera* hypopharyngeal gland. *Proc. 6th Int. Congr. IUSSI, Bern* : 121-130.

Noirot, C. & A. Quennedey (1974). Fine structure of insect epidermal glands. *Ann. Rev. Entomol.,* 19 : 61-80.

Reimann, K. (1953). Untersuchungen an der Thoraxdrüse der Arbeiterin von *Apis mellifica*. *Naturwissenschaften*, 40 : 538.

Schönitzer, K. & P. Seifert (1990). Anatomy and ultrastructure of the salivary gland in the thorax of the honeybee worker, *Apis mellifera* (Insecta, Hymenoptera). *Zoomorphology,* 109 : 211-222.

Snodgrass, R.E. (1956). *Anatomy of the Honeybee*. Cornell University Press, Ithaca, New York (334 pp).

Belg. J. Zool., 134 (supplement 1) : 61-66 March 2004

Miscellaneous features of electroreceptors in *Gnathonemus petersii* (Günther, 1862) (Pisces, Teleostei, Mormyriformes)

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ABSTRACT. The cutaneous electrical activity of ampullary and tuberous electroreceptor organs of *Gnathonemus petersii* (Teleostei, Mormyriformes) was studied in seven specimens. The spontaneous activity levels of 38 ampullary primary afferents showed significant differences between individual fishes rather than between different loci within a single specimen. Spontaneous activity of ampullary electroreceptor organs is not constant, but depends on some unknown factor, e.g. occlusion of the ampullary opening or local accumulation of ions. Both under Saffan and Hypnodil anesthesia it proved possible to record ampullary primary afferent activity from anesthetized specimens. Ampullary organ activity is modulated by Electric Organ Discharges and by respiratory potentials. Respiratory potentials also modulate the cutaneous electrical activity of mormyromasts. The ion composition of the water influences the cutaneous electrical oscillations of the Knollen organs.

KEY WORDS : Ampullary electroreceptor organ, conductivity, electroreception, *Gnathonemus petersii*, Knollenorgan, mormyromast, tuberous organ.

INTRODUCTION

Electroreceptor organs of electrosensitive fish are usually divided into several categories with different functions or properties : tuberous and ampullary organs (Moller, 1995; Kramer 1996).Tuberous organs are used for the detection of electric high-frequency (300<f<10000 Hz) fields caused by Electric Organ Discharges (EOD's). Within the group of tuberous organs the Knollenorgans of the mormyriformes are used for the detection of electric fields caused by EOD's of other fish, whereas the mormyromasts are used for the detection of distortions of the electric fields caused by the fish's own EOD. Ampullary electrosensory organs, on the other hand, are used for the detection of low-frequency (0.1<f<100 Hz) electric fields not caused by EOD's. These fields emanate from a variety of sources including all aquatic organisms and bottom structures of the environment (Kalmijn, 1972). In the marine environment ampullary organs are centimetre-long jelly-filled canals, the so called ampullae of Lorenzini. In the freshwater environment ampullary organs are only small skin-deep invaginations called microampullae. The present paper deals with freshwater ampullary and tuberous organs.

Earlier studies on catfish ampullary electroreceptor organs in *Clarias gariepinus* revealed that the level of spontaneous activity is related to the topology of the receptor organs. Ventral ampullary organs have lower resting discharges than dorsal ones. Not much is known about the cause of this phenomenon (Peters & Mast, 1983). Originally it was thought that the firing rate of ampullary organs was related to the number of receptor cells that converged to the same single primary afferent. However, this was not the case (Peters & Mast, 1983; Peters et al., 1997; Teunis et al., 1990). It might very well be that, for instance, the type of innervation somehow determines the resting discharge rate.

The aim of the present pilot study was to investigate if, in *Gnathonemus* ampullary organs, a similar place-related level of spontaneous activity could be found. In addition some cutaneous recordings of non-ampullary, tuberous organs were made.

MATERIAL AND METHODS

The experiments were performed on seven specimens of *Gnathonemus petersii*, varying in length from 11 to 18 cm (TL), individually kept in full glass containers of about 20 l in the aquarium of the LPN-CNRS, Gif-sur-Yvette France. The temperature was kept between 21 and 25 °C. The composition of the (Paris tap) water was, in mM : Mg 0.132, Si 0.099, Na 0.826, Ca 1.62, K 0.084, P 0.0, S 0.274, Zn 0.015 (data provided by M. Veron). The conductivity of the tap water was 331 ± 12 µS/cm at 25 °C and pH 6.3. That of the tank water was 422 µS/cm at 25 °C and pH 5.8.

Fish were first anesthetized by immersion in Saffan (Serum- und Impfinstitut, Basel) 2 mg/l for induction, and 1.5 mg/l for maintenance of narcosis. Then they were transferred to a 31 x11.5 x 5 cm perspex tray for mechanical fixation and artificial respiration at 30 to 100 ml/min. Electrical conductivity of the circulating water (taken from the stock tank) including the anesthetic was 422 µS/cm. On two occasions Hypnodil was used as an anesthetic

in a concentration of 30 mg/l for induction, and 5 mg/l for maintenance. For local application of water with a different ion composition to the skin, a ring of putty, inner diameter 1 cm, was placed on the skin.

Recording the activity of an electroreceptor organ was done with tungsten needle electrodes, insulated apart from the tip, and a PAR 113B amplifier with a TEAC cassette recorder. Tuberous organs were recorded at a bandwidth of 300<f<10.000 Hz; ampullary organs were recorded at 300<f<3000 Hz. The ground electrode was placed near the tail of the fish.

In order to study the effect of ion composition of water on electroreceptor organ functioning, three solutions were made with equal conductivity but with different ion contents. We used either normal local tap water, or diluted KCl, or diluted fish Ringer's (Wolf, 1963). Conductivity of water was 331 ± 12 µS/cm at 24.8 ºC and pH 6.2. The conductivity of the isoconductive KC-solution was 342 ± 11 at 21 ºC and pH 6.3. The conductivity of the isoconductive Ringer's solution was 335 ± 14 µS/cm at 21 ºC and pH 6.4.

In order to visualize electroreceptor organs, we incidentally applied a 1% neutral red solution in tap water in the ring on the skin for some minutes.

Differences between dorsally and ventrally situated ampullary organs in two specimens were tested with Kruskal-Wallis non-parametric test for independent samples.

RESULTS

Recognition of electroreceptor organs

It proved rather difficult to identify beyond doubt the three classes of electroreceptor organs by means of visual inspection and electrophysiological recording. It is true that there were small, large and intermediate organs, and that the large organs or Knollenorgans were pinkish as described by earlier investigators (Bennett, 1965; Bennett, 1967; Szabo, 1974; Szabo & Fessard, 1974), but to the inexperienced eye there was an overlap between the classes. According to Jean-Pierre Dénizot (LPN-CNRS) application of the dye toluidine blue stains the ampullary organs blue and the tuberous organs reddish, but we refrained from applying this dye because we feared that the stain would affect functioning. It is also true that ampullary organs give 'regular' spontaneous activities with more bass components than do the tuberous organs when the recordings are made audible via a speaker. The sound from tuberous organs is of a higher frequency. Moreover they tend to oscillate after being touched by an electrode, or if the water height and water composition changes, or if the resistive load on the organs changes.

At the start of the experiment it was very difficult to find functioning ampullary organs. The candidates, the small organs, did not yield spikes. Not even after administering distilled water, which increases the amplitude of the recorded spikes, could activity be recorded (Fig. 1). Eventually we discovered that mechanically opening the ampulla pore with the electrode tip made recording possible.

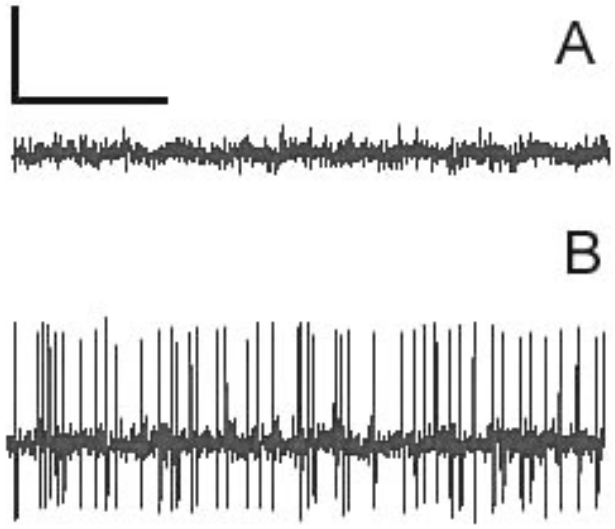

Fig. 1. – Demonstration of the lack of response in ampullary electroreceptor organs, compared to the cutaneous activity recorded from a Knollenorgan. A : Cutaneous electrical activity of an ampullary organ submersed in distilled water. B : Cutaneous electrical activity of a Knollenorgan submersed in distilled water. Scale markers : 100 µV, 50 ms.

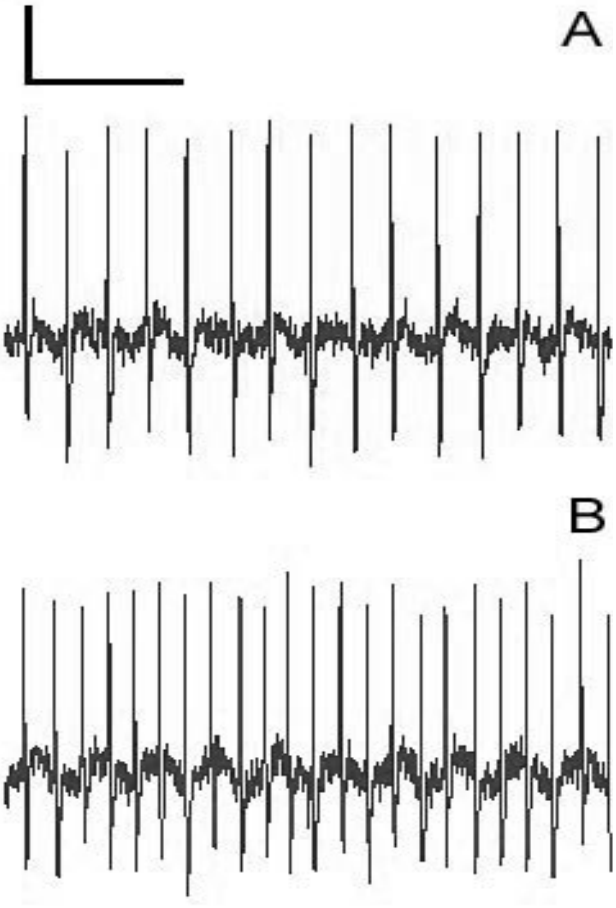

Fig. 2. – Cutaneous activity recorded from ampullary electroreceptor organs in specimens of *Gnathonemus* anesthetized with Saffan (A) 1.5 mg/l, and Hypnodil (B) 5 mg/l. Scale markers : 100 µV, 50 ms.

Anesthetics : Saffan and Hypnodil

Both under Hypnodil- and Saffan anesthesia it proved possible to record activity from the pores of the ampullary organs (Fig. 2). The effect of both anesthetics on sensory function was not rigorously investigated. Both under Hypnodil and Saffan anesthesia the Electric Organ Discharges (EOD) continued to some extent. Most likely the depth of anesthesia plays a part here.

Recording of ampullary organs

Probing with the electrode into the ampulla pore resulted in a high frequency series of spikes, Once 'opened', the ampulla continued to fire until, after some minutes, it sometimes stopped firing, but the recorded spikes did not change in amplitude. An example of such a recording is given in Fig. 3. The spontaneous activity of 38 ampullae was recorded at six spots, in two fishes. The sites were dorsally and ventrally between the dorsal fin and snout, and on the snout. The Kruskal-Wallis test revealed no differences between dorsally- and ventrally-situated ampullary organs (p=0.92), but a significant difference between the two specimens (p=0.003). Average interspike interval lengths of more than 800 intervals for each ampulla are given in table 1. Further, the ampullary organs responded to ventilatory movements of the operculum, if the fish recovered from anesthesia (Fig. 4). Also the EODs caused strong modulations of the spontaneous activity (cf. Bell & Russell, 1978)(Fig. 5).

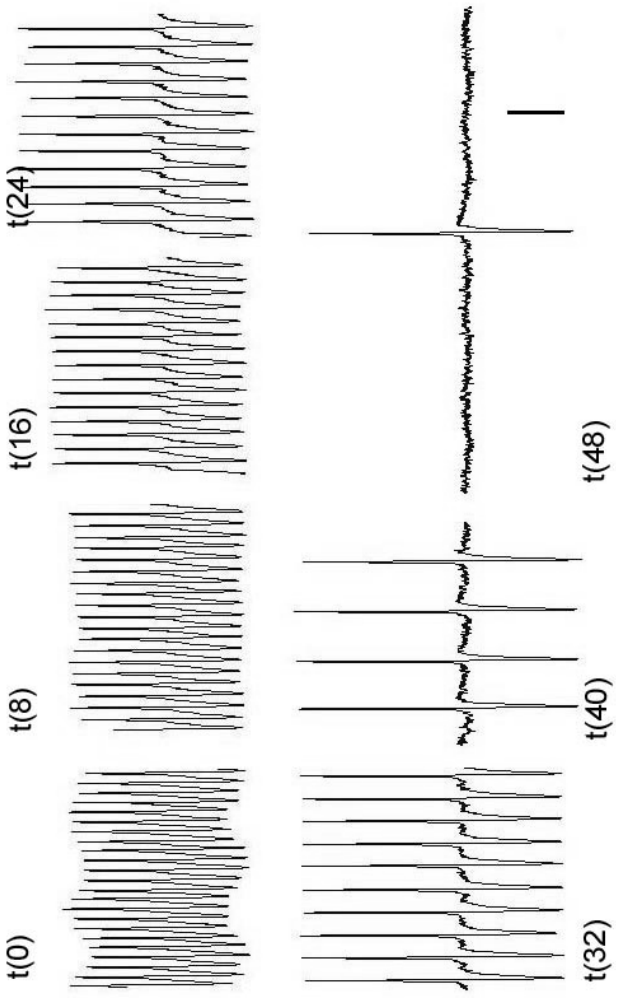

Fig. 3. – Cutaneous activity recorded from an ampullary electroreceptor organ after opening its pore with a tungsten needle electrode. The blocks each represent 50 ms recordings, made every 8 s. Before opening the pore the ampullary organ is 'silent'. After opening it starts with a high frequency spontaneous activity that slows down and eventually stops. The spikes remain large, indicating that the ampullary pore is still open. Scale marker : 200 µV.

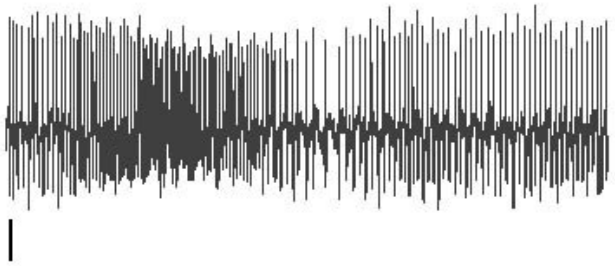

Fig. 4. – Cutaneous activity recorded from an ampullary electroreceptor organ, modulated by ventilatory potentials. The whole recording lasts about .9 s, and corresponds roughly to 2/3 of a respiratory cycle. *Gnathonemus* recovering from affan anesthesia. Scale marker : 100µ.

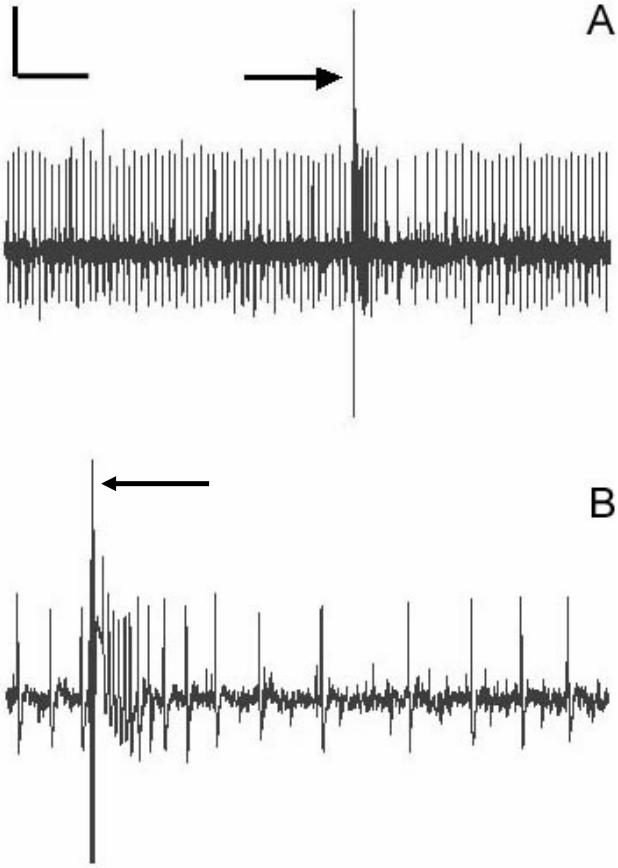

Fig. 5. – Cutaneous activity recorded from an ampullary electroreceptor organ, modulated by an Electric Organ Discharge, while *Gnathonemus* is recovering from Hypnodil anesthesia. Scale markers : 200 µV, 150 ms (A), and 30 ms (B). Arrow : Electric Organ Discharge.

TABLE 1

Average interspike intervals of spontaneous activity of ampullary electroreceptor organs in *Gnathonemus petersii*

Ampullae, Spontaneous activity	Average interspike intervals of n ampullary organs in fish 1 (ms)	Average interspike intervals of n ampullary organs in fish 2 (ms)
Dorsal	15 ± 7 S.D. (n = 8)	9 ± 6 S.D. (n = 6)
Ventral	13 ± 4 S.D. (n = 9)	9 ± 5 S.D. (n = 15)

Recording of tuberous organs, i.c. Knollenorgans

The resistive load, i.e. the conductivity of the water, determines the amplitude of the recorded cutaneous signals. After administration of a Ringer's solution in a putty ring, no spikes could be recorded, whereas after administration of distilled water the amplitude of the spikes increased about tenfold (Fig. 6).

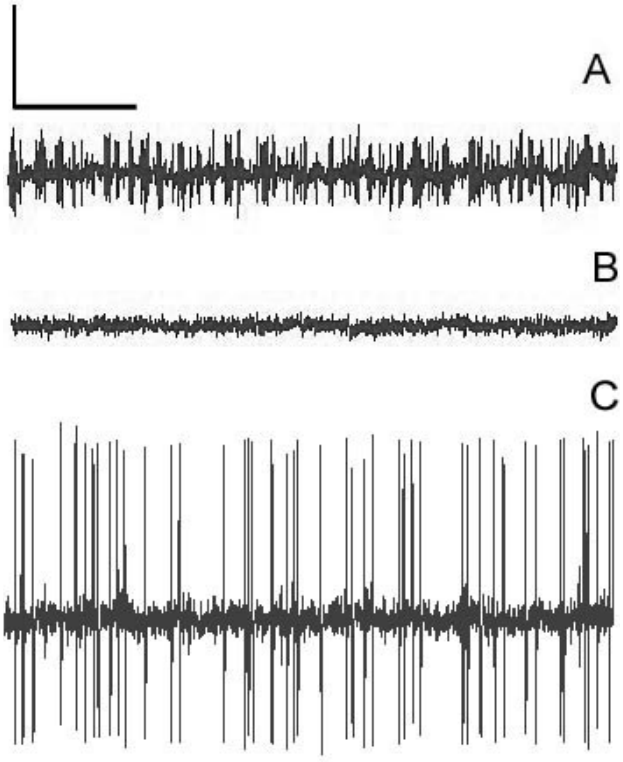

Fig. 6. – Cutaneous activity recorded from a Knollenorgan in three media with different electrical conductivities. A : tap water; B : Ringer's solution; C : Distilled water. Scale markers : 200 μV, 200 ms.

Touching the Knollenorgans with an electrode can evoke ringing i.e. oscillations. We found it was not necessary to dry the skin as mentioned by previous authors (Bennett, 1965; Bennett, 1967; Szabo, 1962; Szabo, 1963; Szabo & Fessard, 1965). Even with 3 cm of water over the skin, ringing could be recorded at 1 cm distance from the skin. Administration of 1% neutral red on the skin and subsequent rinsing resulted in patches of brown precipitate more or less over the tuberous organs (Fig. 7).

Since earlier studies in catfish ampullary electroreceptors revealed that they are very sensitive to changes in the water composition (Roth, 1971), we also tested how the ion composition of the water influenced Knollerorgan functioning. We therefore changed the water within a circular ring placed on the skin around an organ. We took care to match the electrical conductivities. Surprisingly the water composition changed the oscillations of the Knollenorgans (Fig. 8). When normal tap water was administered the 'ringing' activity disappeared, whereas it reappeared when Ringer's or KCl solutions were applied. Further respiratory movements proved to modulate the firing or oscillation frequency of tuberous organs, i.e. mormyromasts (Fig. 9).

DISCUSSION

Anesthesia

Both Saffan and Hypnodil were satisfactory anesthetics for recording the cutaneous activity of the electroreceptor organs, apparently because electroreceptor organs do not contain GABA membrane receptors, which are the target of both anesthetics (Peters et al., 2001; Grant et al., 1999).

Ampullary organs

The recordability of the ampullary activity seems to depend on the 'openness' or 'closedness' of the pores. In freshwater catfish this phenomenon is not seen to this extent. The course of the frequency change (Fig. 3) demonstrates that it is not the openness of the ampulla per se that influences recordability. The end of the recording in Fig. 3 shows that the amplitude of the spike remains large, whereas the frequency drops. So the electrical recording condition did not change. It is not clear how this phenomenon should be interpreted. Perhaps the spontaneous activity of the ampullary organs depends somehow on the water composition. The stock tank water must have been rather polluted since its resistivity amounted to 442 μS/cm versus Paris tapwater 332 μS/cm. Earlier it was demonstrated that the water composition influences both spontaneous activity and evoked activity in catfish ampullary organs (Roth, 1971; Peters & Westerink, 1999). The spatially related differences of spontaneous activity found in catfish ampullary organs were not seen in *Gnathonemus*.

Knollenorgans and mormyromasts

The Knollen organs and mormyromasts behaved as described by the early pioneers (Bennett, 1965; Bennett, 1967; Szabo, 1962; Szabo, 1963; Szabo & Fessard, 1965; Szabo, 1967; Szabo, 1974; Szabo & Fessard, 1974). Conspicuous is perhaps that respiratory movements, and consequently respiratory potentials (cf. Kalmijn, 1972), modulate the activity of mormyromasts. Further the composition of the surrounding water also has a strong effect on the cutaneous activity of Knollenorgans. It is possible that the diffusion potentials (liquid junction potentials) of the different fluids modulate the activity. On the other hand it is likely that the ionic composition somehow influences the electrochemical homeostasis of the receptor cells and thus causes ringing. It is generally thought that the path from receptor cells to the

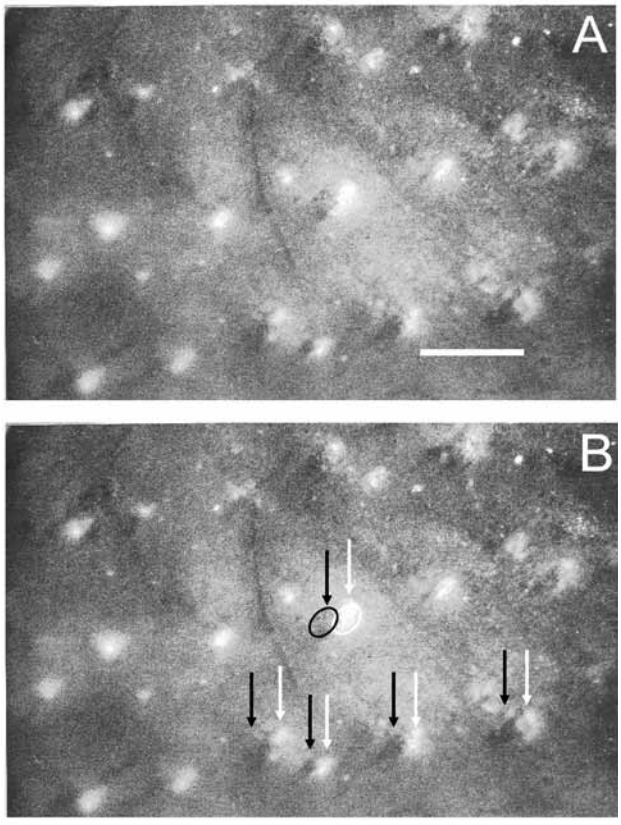

Fig. 7. – Photograph of tuberous electroreceptor organs after exposure to 1% neutral red solution. A : Plain photograph. B : Photograph with markers. Note the dark spots representing precipitated neutral red (black oval, black arrow) covering half of the unpigmented electroreceptor organs (white oval, white arrow). Scale marker : 1mm.

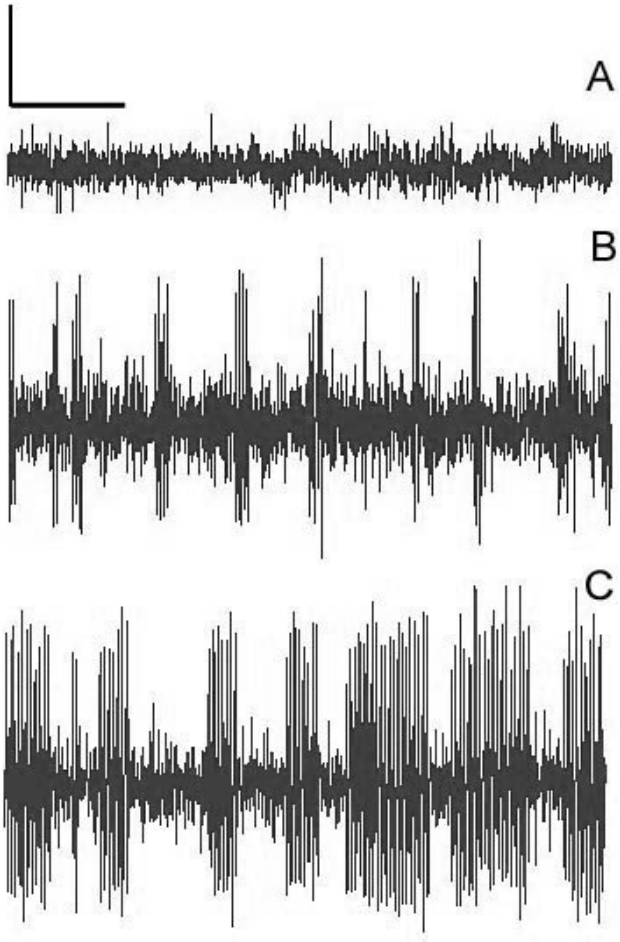

Fig. 8. – Cutaneous activity recorded from a Knollenorganorgan, in 3 solutions with equal conductivities but with different ionic compositions. A : normal water. B : Ringer's solution. D : Diluted KCl. Conductivity of all solutions 335 ± 14 µS/cm. Scale markers : 50 µV, 50 ms.

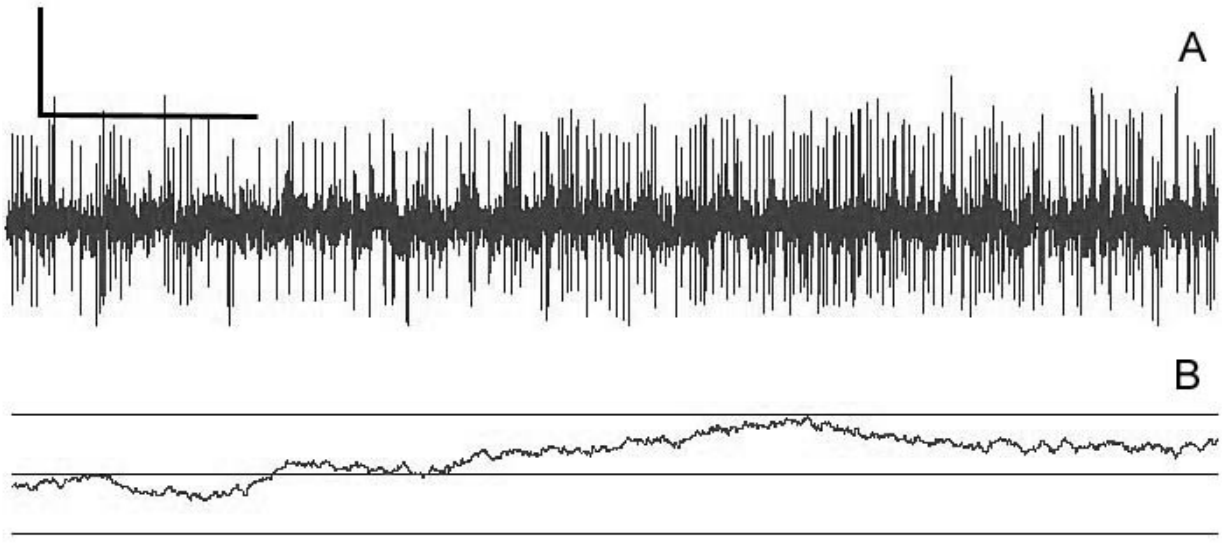

Fig. 9. – Cutaneous activity recorded from a mormyromast, modulated by ventilatory potentials. The right half of the recording shows an increased activity with respect to the left half. A : cutaneous recording. B : 'integrated signal' as running average (n=3000) of the absolute values of A. The whole recording lasts about .6 s, and corresponds to less than a respiratory cycle. Scale markers 50 µV/cm, 100 ms.

outer world is blocked by a capacitor, such as cell membranes, but there are also indications for a resistive path. External application of a 1% neutral red solution to the skin causes brown precipitates near the plug of the tuberous organ (Fig. 7). Such precipitates are, among other things, caused by high concentrations of ions (unpublished tests). This could mean that the plug of the tuberous organ is much more leaky than thought hitherto. In that case the ionic composition could influence the functioning of the tuberous organs either by liquid junction potentials or by the ion-mix.

Conclusion

We conclude from the data mentioned above that there is no position-related difference in the spontaneous afferent rate of the ampullary organs, and that the water composition plays an important part in the functioning of both ampullary and tuberous receptor organs.

ACKOWLEDGEMENTS

The experiments were performed during a one week stay at the Laboratoire de Physiologie Sensorielle at CNRS, Gif-sur-Yvette, France in 1990. The hospitality of the late Thomas Szabo, Kirsty Grant, Jean-Pierre Dénizot, Jacques Serrier and Michele Veron is gratefully acknowledged, as well as the critical remarks of two anonymous referees.

REFERENCES

Bell, C.C., & C.J. Russell (1978). Effect of electric organ discharge on ampullary receptors in a mormyrid. *Brain Res.,* 145 : 85-96.

Bennett, M.V.L. (1965). Electroreceptors in Mormyrids. In : *Sensory receptors*. Frisch, L. (Ed.). Cold Spring Harbor symposium on quantitative biology (30; 1965; Cold Spring Harbor) : 245-262.

Bennett, M.V.L. (1967). Mechanisms of electroreception. In : *Lateral line detectors*. Cahn, P.H. (Ed.). Indiana University Press, Bloomington : 313-393.

Grant, K., G. Von Der Emde, L.G. Sena & C. Mohr (1999). Neural command of electromotor output in mormyrids. *J. Exp. Biol.,* 202 : 1399-1407.

Moller, P. (1995). *Electric fishes, history and behavior.* Chapman & Hall, London (584 pp.).

Kalmijn, A.J (1972). Bioelectric fields in sea water and the function of the ampullae of Lorenzini in elasmobranch fishes. *SIO Ref.,* 72-83 :1-21.

Kramer, B. (1996). Electroreception and communication in fishes. *Progr. Zool.* 42, Gustav Fischer Verlag, Stuttgart.

Peters, R.C., R.J. Brans, F. Bretschneider, E. Versteeg & A.Went (1997). Converging electroreceptor cells improve sensitivity and tuning. *Neurosci.,* 81 : 297-301.

Peters, R.C.& F. Mast (1983). Information processing by ampullary receptors : parallel convergent inputs. *Comp. Biochem. Physiol.,* 76A : 143-151.

Peters, R.C., B. Van den Hoek, F. Bretschneider & M. L. Struik (2001). Saffan : a review and some examples of its use in fishes (pisces : teleostei). *Neth. J. Zool.,* 51 : 1-17.

Peters, R.C. & R.H.S. Westerink (1999). Catfish electroreceptor organ functioning during five days exposure to different calcium environments. *Fish Physiol. Biochem.,* 21 : 81-88.

Roth, A. (1971). Zur Funktionsweise der Elektrorezeptoren in der Haut von Welsen (*Ictalurus*) : Der Einfluss der Ionen im Süsswasser. *Z. vergl. Physiol.,* 75 : 303-322.

Szabo, T. (1962). Spontaneous electrical activity of cutaneous receptors in mormyrids. *Nature,* 194 : 600-601.

Szabo, T. (1963). Elektrorezeptoren und Tätigkeit des elektrischen Organs der Mormyriden. *Naturwiss.,* 50 : 447-449.

Szabo, T. (1967). Activity of peripheral and central neurons involved in electroreception. In : *Lateral line detectors*. Cahn, P.H. (Ed.). Indiana University Press, Bloomington : 295-311.

Szabo, T. (1974). Anatomy of the specialized lateral line organs of electroreception. In : *Electroreceptors and other specialized receptors in lower vertebrates*.. Fessard, A. (Ed.). Springer, Berlin : 13-58.

Szabo, T. & A. Fessard (1965). Le fonctionnement des électrorécepteurs étudié chez les Mormyres. *J. Physiol. (Paris),* 57 : 343-360.

Szabo, T. & A. Fessard (1974). Physiology of electroreceptors. In : *Electroreceptors and other specialized receptors in lower vertebrates*. Fessard, A. (Ed.). Springer, Berlin : 58-124.

Teunis, P.F.M., F. Bretschneider & R.C. Peters (1990). Convergence ratio and transduction in catfish electroreceptive organs. *Comp. Biochem. Physiol. A.,* 97 : 405-410.

Wolf, K. (1963). Physiological salines for fresh water teleosts. *Progr. Fish. Cult.,* 25 : 135-140.

Telestacicola xenophiothricis n. sp. (Copepoda, Poecilostomatoida), a remarkably well adapted commensal of the brittlestar *Ophiothrix purpurea* (Echinodermata)

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ABSTRACT. A study of the turbellarian commensal fauna of the brittlestar *Ophiothrix purpurea* in Hansa Bay (Papua New Guinea) revealed the presence of a remarkably well-adapted lichomolgid copepod : *Telestacicola xenophiothricis* n. sp. (Rynchomolgidae, Poecilostomatoida). Fourteen out of 118 (12%) ophiuroids inspected were infested by one to three copepods (a total of 18 specimens observed). The body colour of this commensal crustacean mimics perfectly the pattern of orange-red and pink lines of the central disk of the host, and this complex and uncommon mimicry could indicate a high host specificity. *T. xenophiothricis* is the fourth member in this genus, but is the first encountered on ophiuroids (Echinodermata), while it is the tenth species of Poecilostomatoida amongst the 46 known species of Copepoda associated with ophiuroids (others are 22 Siphonostomatoida, 13 Cyclopoida and one Harpacticoida). Furthermore, the generic assignment of *Doridicola claudus* Humes & Stock is discussed, and the species is allocated to the genus *Telestacicola*.

KEY WORDS : Copepoda, new species, host preference, mimicry, Papua New Guinea.

INTRODUCTION

Since the establishment of the first lichomolgid genus *Doridicola* by Leydig in 1853, our knowledge about the lichomolgid complex has expanded tremendously (Humes, 1994). In recent years two basic revisions of this taxon have been published (Humes & Stock, 1973; Humes & Boxshall, 1996), inclusive in the latter, a phylogenetic analysis of the relationships between the several families. Today, the lichomolgid complex comprises ten families with hundreds of species distributed over 122 genera. Despite this exponential growth of our knowledge of the species richness of this group, the cited figures certainly represent only the tip of the iceberg (Humes, 1994). However, where we have now a sound systematic framework for this superfamily, the study of their behaviour and their impact on the hosts is a vast open field of research (Gooding, 1957; Humes, 1982).

During a study of the symbiont polyclad *Discoplana* spec. (Turbellaria) in the brittlestar *Ophiothrix purpurea* von Martens, 1867 (Ophiotrichidae, Echinodermata), an interesting lichomolgoid copepod was detected living on the central disc of the host. The present contribution deals with the description of this new species, with some information on its behaviour and its remarkable colour pattern.

MATERIAL AND METHODS

Ophiuroids *Ophiothrix purpurea* von Martens, 1867 were collected from the coral reefs surrounding Laing Island (4˚10'S, 144˚52'E), Hansa Bay and Durangit Reef (Madang Province, Papua New Guinea). All specimens were collected by Scuba diving between 3 to 20 m deep during three missions performed between 1994 and 1996. During collections, echinoderms were placed individually in plastic bags. Once in the laboratory at the Biological Station King Leopold III on Laing Island, they were immediately observed and dissected under a stereomicroscope. Copepods found were counted, photographed and fixed in 100% ethanol or in Bouin's fluid for morphological studies. After 24h, the latter were transferred to 75% ethanol for preservation.

The dissected specimens were mounted in glycerine with Euparal®-sealed cover glasses. Preserved specimens were stored in 75% ethanol. Observations were made on a Leitz Diaplan light microscope equipped with phase contrast and a drawing tube. Descriptive terminology follows Humes & Boxshall (1996). All specimens were incorporated in the Invertebrate Collections of the Royal Belgian Institute of Natural Sciences (labeled COP).

SYSTEMATICS

Family Rynchomolgidae Humes & Stock, 1973
Telestacicola xenophiothricis n. sp.

Material : Holotype : 1 dissected female, labeled COP 4580A-E; allotype : 1 dissected male, labeled COP 4581A-D; paratypes : 1 dissected female (COP 4582A-

B); 2 females and 1 male, preserved in alcohol, labeled COP 4583.

Type locality : Lagoon of Laing Island, Madang Province, Papua New Guinea.

Host : Ophiothrix purpurea von Martens, 1867 (Echinodermata : Ophiotrichidae).

Etymology : The specific epitheton, *xenophiothricis*, is a conjunction of the Greek words *Xeno*, meaning guest, and *Ophiothrix*, brittlestar.

Description

Female

Body (Figs 1A & B) graceful cyclopoid shaped, 1.75 mm long (including caudal rami, but without setae). Head with dorsal trace of separation between head and first pedigerous somite. Third and fourth pedigers slightly expanded laterally.

Genital double somite expanded laterally in anterior part (190 μm wide) and nearly cylindrical in posterior half (115 μm wide). Length of somite : 210 μm. Leg 6 with setiform lateral element (± 45 μm long) and two spiniform processes. Valves ovate. The three postgenital somites are (LxW, in μm) 67x90, 67x80, 100x73. Leg 5 bearing somite 163x86 μm, with lateral seta 38 μm long. Dorsal integument with pattern of minute perforations in proximal half.

Caudal rami cylindrical, 96 x 16 μm (6.4/1) with six setae. Dorsal seta 28 μm long, proximal lateral seta 56 μm, outer terminal seta 150 μm, and median terminal seta 170 μm long. Proximal lateral and dorsal setae smooth, all others feathered. Inner terminal seta and distal lateral one broken.

Antennule (Fig. 2A) 7-segmented. Segments with following lengths (measured along median axis) : 55, 85, 30, 45, 50, 40, 40. Setal complement (Aesth. means aesthetasc) : 4-13-6-3-5-2+Aesth-7+Aesth.

Antenna (Figs. 3A, B & C) 4-segmented with following armature : 1, 1, 3, 5+2. First endopodal segment with field of spinules in proximal half and row of spinules along inner margin. Terminal segment spinulose along inner margin. Elements of third segment long, reaching far beyond middle of fourth segment. Claws with pinnate proximal half, and serrate distal one.

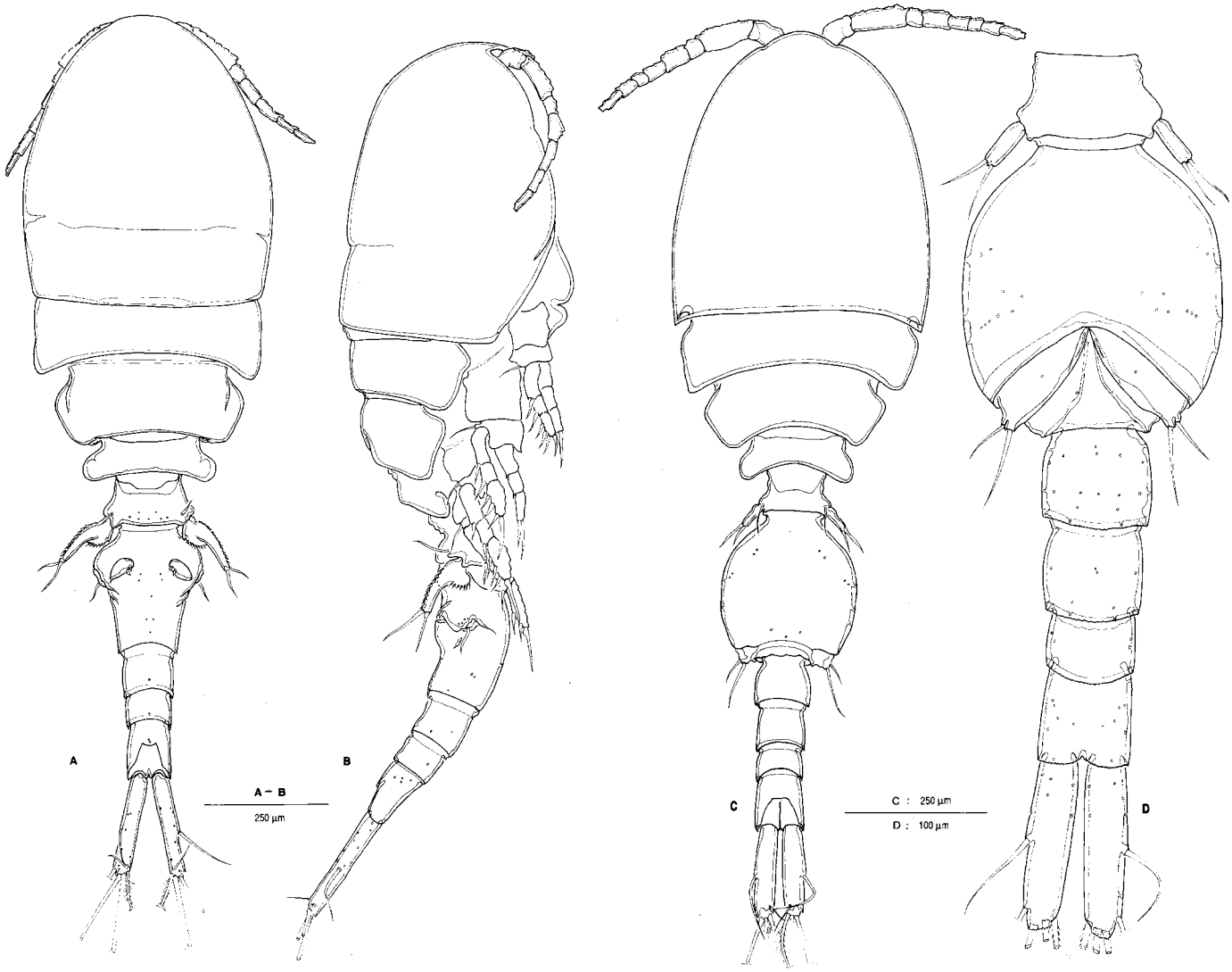

Fig. 1. – *Telestacicola xenophiothricis* n. sp., female. A, Habitus in dorsal view; B, Habitus in lateral view; Male. C, Habitus in dorsal view; D, Urosome, in ventral view.

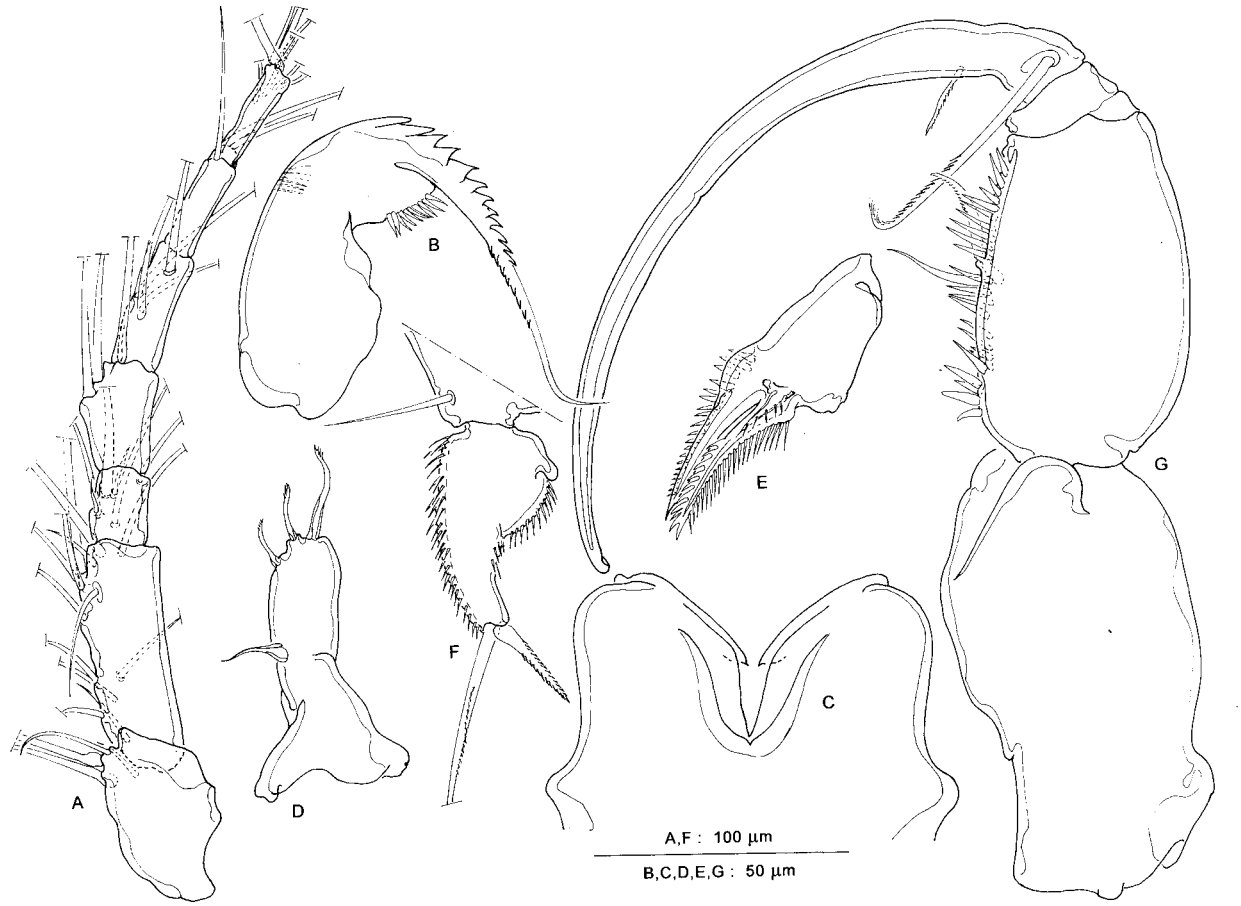

Fig. 2. – *Telestacicola xenophiothricis* n. sp., female. A, Antennule; B, Mandible; C, Labrum; D, Maxillule, E, Maxilla; F, Leg 5; Male : G, Maxilliped.

Mandible (Fig. 2B) with slender lash, having 12 medial teeth (those along the convex side) and eight teeth in the middle of the concave side. Maxillule (Fig. 2D) short, with four setae. Lateral seta thickened at base. Terminal 3 setae robust, pinnate apically. Maxilla (Fig. 2E) with stout, unarmed, basal segment (not drawn). Distal segment attenuated into short lash armed with about 20 teeth. Auxiliary lash with nine teeth along outer margin, and densely armed with long and slender spinules along the entire medial margin. Additional seta short, arising near insertion of auxiliary lash. Maxilliped (Fig. 3D) with robust basal segment devoid of elements. Second segment twice as long as wide with two setae at midlength of medial margin. Terminal segment short with two terminal claw-like confluent elements.

Legs 1-4 with large square and smooth coxa. Outer distal region somewhat protruded and medial distal edge with feathered seta. Intercoxal sclerite in all legs with smooth surface. Basis of all legs with short and naked outer seta. Medial margin of basis rounded and smooth. Rami 3-segmented except for the fourth leg endopod, which is one-segmented (Fig. 3I). Distal outer edge of terminal exopodal segment of legs 2-4 protruded into a small hyaline lobe with a bifid distal margin (Fig. 3H). Endopodite of leg 4 just reaching to the middle of the second exopodal segment. Proximal part slightly expanded laterally. Inner lateral seta pinnate, terminal elements spiniform (Fig. 3E). Inner terminal one 51 µm, outer one 38 µm (outer/inner : 0.74/1).

Leg 5 (Fig. 2F) with inflated proximal part and subsquarish distal half. Inner margin of proximal part with a distinct supplementary notch. Inner and outer margin of segment densely ornamented with rigid spines. Outer terminal setiform, broken in all specimens, but at least 2.5 times longer than inner spiniform element.

Male

Habitus (Fig. 1C) cyclopoid, 1.25 mm long. Cephalothorax without trace of separation between head and first pedigerous somite. Third and fourth pedigerous somite slightly expanded laterally. Second urosomal somite large and ovate (194x195 µm), bearing well-developed sixth legs. Third to last urosomal somites narrow, respectively (LxW, in µm) : 69x77, 66x69, 39x61, 69x61.

Caudal rami 3.8 times as long as wide, with six setae. Ventrodistal margin slightly protruded, and serrate. Proximal lateral seta (75 µm) arising in middle of outer margin, distal lateral one implanted close to distal outer edge. Dorsal seta 75 µm long, located near distal inner edge. Outer distal and all terminal setae broken.

Antennule 7-segmented with following complement : 4 – 12+Aesth. – 6 – 3+Aesth – 4+Aesth – 2+Aesth – 7+Aesth.

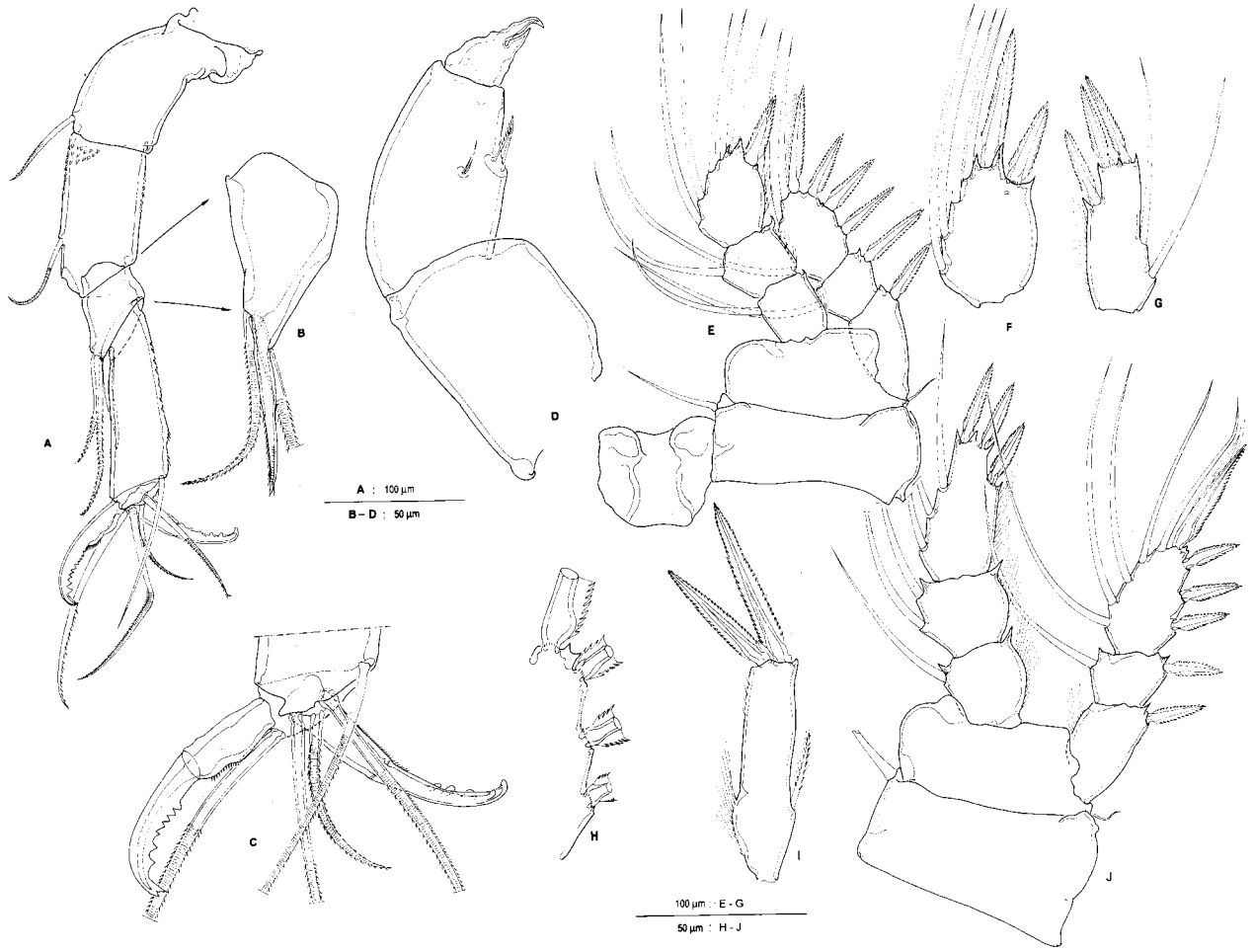

Fig. 3. – *Telestacicola xenophiothricis* n. sp., female. A, Antenna, B, Third antennal segment, enlarged; C, Distal margin of terminal antennal segment, enlarged; D, Maxilliped; E, Leg 1; F, Distal endopodal segment of male leg 1; G, Distal endopodal segment of leg 2; H, Detail of distal exopodal segment of leg 2; I, Leg 4 endopodite; J, Leg 3.

Maxilliped (Fig. 2G) with long, unarmed basal segment. Second segment with long ovate appearance, densely armed with robust spinules along the medial margin, and bearing two elements near the middle of the medial margin. Third segment small, unarmed. Claw long, blunt and with two elements. Proximal one four times longer than distal one.

Leg 1 (Fig. 3F) typically sexually dimorphic : terminal endopodite segment with four medial setae, one terminal and one subdistal outer spine.

Leg 5 (Fig. 1D) short (36x12 µm) and cylindrical, bearing two subequal setiform elements : outer one 44 µm, inner one 38 µm. Leg 6 (Fig. 1D) as a triangular plate with two setiform elements : outer one 36 µm, inner one 58 µm.

Affinities

Within the key to the genera of the Rynchomolgidae (Humes & Boxshall, 1996), the specimens at hand key out to the genus *Telestacicola* Humes & Stock, 1973 in which nowadays three species are assembled : *T. angoti* Humes & Stock, 1973 (type-species), *T. sertus* Humes, 1977 and *T. lobophyti* Humes, 1990. *T. xenophiothricis* n. sp. is at once distinguishable from *T. lobophyti* by the longer caudal rami in the male (3.8/1 versus 3.17/1) and female (6.5/1 versus 4.8/1), the serrate antennal claws, and the much longer and wider articulating segment of the fifth leg. *T. sertus* differs from the here described species by its short caudal rami (only 1.6 times longer than wide) and the dentate nature of the antennal claws.

T. xenophiothricis resembles most closely *T. angoti*. Both species share the graceful cyclopid body shape with long caudal rami, a proximally inflated female fifth leg, and the long outer terminal spine on the fourth leg endopodite, reaching far beyond the middle of the inner element. The former has, however, longer caudal rami (female : 6.4/1 versus 4.8/1; male : 3.8/1 versus 3.19/1), and a much more compact endopodite in the fourth leg, reaching only to the middle of the second exopodal segment. *T. xenophiothricis* differs significantly from its congeners in the elements on the antenna. Whereas *T. angoti* has terminal claws, which are serrate along the entire concave part of the stem, *T. xenophiothricis* is only serrate in the distal half of the stem of the claw, whilst the proximal part of the stem is finely dentate. Moreover, the elements on the third antennal segment in *T. angoti* are clearly much shorter than in *T. xenophiothricis* where the three elements reach far beyond the middle of the terminal segment.

The previously known species of this lichomolgid genus have been found as associates on Telastacea (*T. angoti*, *T. sertus*) and Alcyonacea (*T. lobophyti*). *T. xenophiothricis* is the first representative of the genus known to live on an echinoderm. Among the 41 genera recognised within the family, *Telestacicola* is the third genus besides *Critomolgus* Humes & Stock, 1983 and *Doridicola* Leydig, 1853 known to include species that live in association with cnidarians as well as with other invertebrates (Nudibranchia, Porifera, Bivalvia, Gastropoda, Cephalopoda, and Echinodermata) (Humes & Boxshall, 1996; Ho & Kim, 2001).

We cannot ignore the close resemblance between the genus *Telestacicola* and the genera *Doridicola* and *Critomolgus*. As a matter of fact the three genera are only discriminated by the morphology of the fourth leg. *Critomolgus* differs from the two other genera by the (plesiomorph) chaetotaxy of the terminal segment of the exopodite in the fourth leg. With a complement of III,I,5 it differs significantly from its sister taxa (*Doridicola* and *Telestacicola*), which possess a II,I,5 complement on this segment. *Telestacicola* is distinguishable from *Doridicola* because of the one-segmented condition of the endopodite in the fourth leg. Among the 45 species possessing a two-segmented endopodite in the fourth leg and which are consequently attributed to the genus *Doridicola*, one species, namely *D. claudus* Humes & Stock, 1973, has a short and functionally 1-segmented endopodite in the fourth leg. An indistinct and incomplete transversal line is located where the other *Doridicola* species show the articulation between the proximal and distal segments. Without enlarging the generic diagnosis of *Doridicola*, *D. claudus* cannot be maintained in it and should be transferred to *Telestacicola* and referred to as *T. claudus* (Humes & Stock, 1973) comb. nov.

Without doubt the herein described *T. xenophiothricis* and *T. claudus* resemble each other in many characteristics, but the former differs from the latter by its longer caudal rami, the longer armature on the second antennal segment and the serrate nature of the terminal antennal claws.

THE COPEPOD-HOST RELATIONSHIP

In comparison with the three other associates (the polyclad *Discoplana* spec., the polynoid annelid *Hololepidella nigropunctata* and the phyllodocid annelid *Eumida ophiuricola*; Doignon, pers. obs.; Britayev et al., 1999) of which the first two showed a much higher infestation rate of the host in the study area, *T. xenophiothricis* was found only in one station in some abundance. In this station, 11 out of 30 host specimens (37%) were infested with the copepod. In the other three stations infestation rates never exceeded 4%.

In most cases only a single copepod specimen was detected on the ophiurid central disc, but some brittlestars carried up to three copepod specimens on their body. The copepods seem to be explicitly located on the central disc, as they were never detected on the arms of the host.

The ophiurid host is, like so many of its congeners, a colourful animal with a magnificent and complex pattern of reddish lines (Fig. 4). The animals are commonly encountered aggregated on top of a wide diversity of branched scleractine and alcyonarian corals. Within the study area, *O. purpurea* was found on ten different scleratine corals (Acroporidae, Pocilloporidae and Poritidae) and on one species of Alcyoniidae.

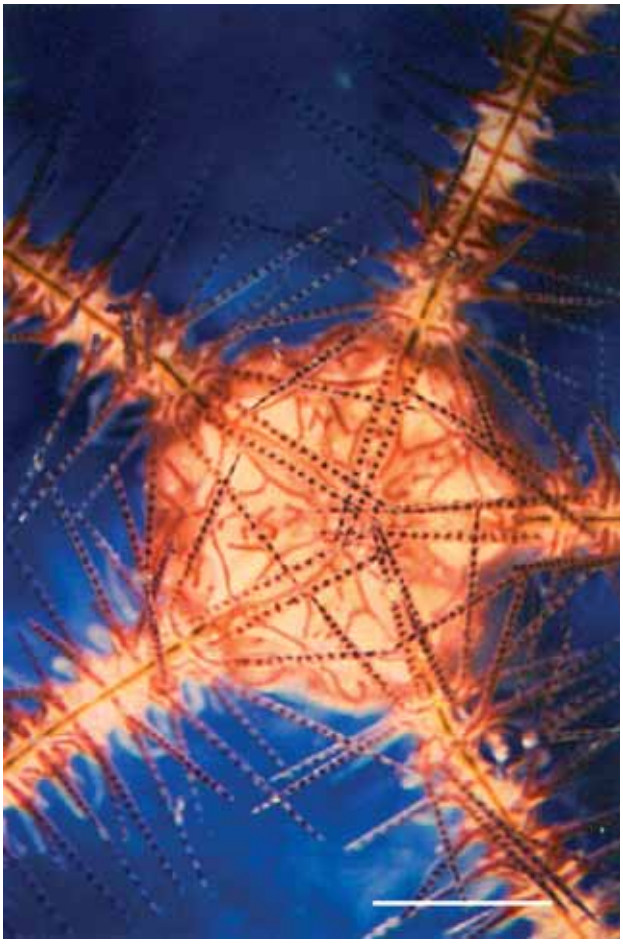

Fig. 4. – Central disc of *Ophiothrix purpurea* von Martens, 1867 showing colour pattern (Scale : 5 mm).

The low infestation rate of the copepod, and the fact that three congeners are known from hydrozoan and anthozoan hosts led at first to the suggestion that the copepods were in fact strays from the populations living on the corals over which the ophiurid was crawling. However, the presence of a copepod associate was not readily detected during the in situ observations of the ophiurid by one of the authors (G.D.). As the copepod body has a particular colouration pattern of reddish lines dorsally and laterally on the prosome and laterally along the urosome on a creamy white background (Fig. 5), it mimics perfectly the colouration pattern of its host. The fact that the copepod remains attached with its buccal pieces fixed on the spiniform projections of the host's disc over a long period of time, made it difficult to locate the animal.

At the dawn of copepodology, researchers were already fascinated by the fact that several copepods living in association with other invertebrates were often colourful, and the bright illustrations, for example, in Canu (1892) are well known among those working in this field of research. The body colour in life of several lichomolgid copepods has been described (Humes & Stock 1973; Humes,

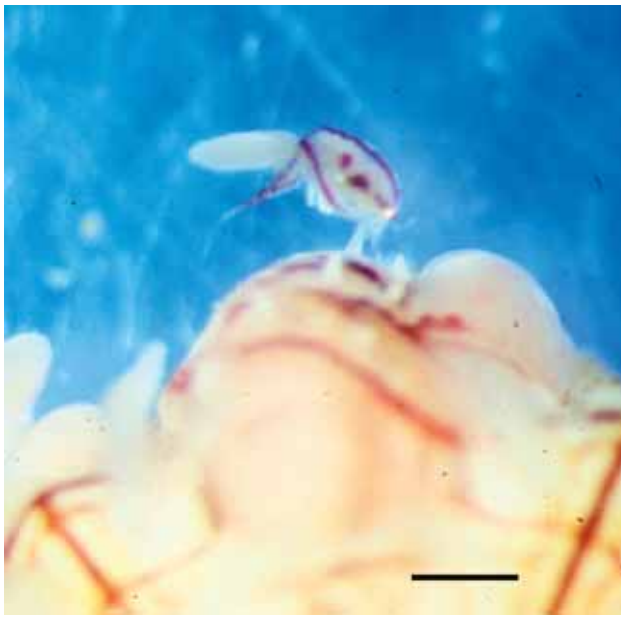

Fig. 5. – Part of the central disc of *Ophiothrix purpurea* with an ovigerous specimen of *Telestacicola xenophiothricis* n. sp. (Scale : 1 mm).

1982; Humes, 1990). If colouration is present, the overwhelming majority have a uniform body colouration (light opaque gray to light brown) with the egg sacs somewhat darker. Others are transparent but have brightly coloured inner organs. *Herrmannella bullata* Humes & Stock, 1973 (Lichomolgidae) living in association with scallops, is in this context remarkable. Scallops show a clearly defined sexually-dimorphic colour of the tissues. Males are deep orange while females are white. According to the sex of the host, the copepod associate mimics the background on which it lives.

Among the Rynchomolgids, the red body color of *Acanthomolgus astrictus* Humes & Stock, 1973 has been said to imitate the color of the host gorgonian on which it lives, and species of the genus *Metaxymolgus* Humes & Stock, 1973 living on certain sea anemones are known to be vividly coloured with a dark red or dark green digestive cross resembling the coloured spots on the column of the host (Humes, 1982).

Apparently the associated copepods, with a total body length of slightly more than 1 mm, may have advantage in their body colouration.

Brittlestars, and Echinodermata in general, are known to provide refuge for a wide array of invertebrates. Jangoux (1990) listed 19 species of copepods parasitic on or in ophiuroids. If we include the species for which the parasitic nature has not been proved (quoted as "commensal" or "associates") and the species described since that review, there are actually 46 species of Copepoda associated with ophiuroids. Most of them are Siphonostomatoida (22 species), which are ectosymbiotic, little modified and generally quite harmless to their hosts (genera *Asterocheres*, *Collocheres*, *Collocherides* and *Ophiurocheres* from the family Asterocheridae (Humes, 1986; Gotto, 1993; Humes, 1998); *Cancerilla*, *Cancerillopsis*, *Ophiopsyllus*, *Parartotrogus* and *Parophiopsyllus* from the family Cancerillidae (Bartsch, 1994; Humes & Hendler, 1999)). *Codoba discoveryi* from the new family Codobidae (Boxshall & Ohtsuka, 2001), the cancerillid *Ophiopsyllopsis indicus* and the asterocherid *Collocherides astroboae* are the only Siphonostomatoida endosymbiotic on ophiuroids, the first two being found in the genital bursae of their hosts and the latter being found in their stomach (Jangoux, 1990; Humes & Hendler, 1999). The Cyclopoida associated with ophiuroids (13 known species) are endosymbiotic, highly modified and induce galls on their hosts (genera *Arthrochordeumium*, *Chordeumium*, *Lernaeosaccus*, *Ophioicodes*, *Ophioika* and *Parachordeumium* from the family Chordeumiidae (Boxshall, 1988; Bartsch, 1996)). The cyclopoid *Thespesiopsyllus* (*Thaumatopsyllus*) *paradoxus*, classified as Monstrilloida for a long time, is reported from the stomach of five species of ophiuroids (Barel & Kramers, 1977; Jangoux, 1990; Huys & Boxshall, 1991). One Harpacticoida, *Thalestris longimana*, is known as ectosymbiotic on *Ophiopholis aculeata* and *Ophiothrix fragilis* (Dahms, 1990). Among Poecilostomatoida, *Telestacicola xenophiothricis* is the tenth known species associated with ophiuroids. With the exceptions of *T. claudus* found in the stomach of *Euryale aspera* and *Critomolgus astrophyticus* occasionally found in the same compartment in *Astrophyton muricatum* (Humes & Stock, 1973; Williams & Wolfe-Waters, 1990), all these poecilostomatoids (genera *Doridicola*, *Presynaptiphilus*, *Pseudanthessius* and *Telestacicola*) are exclusively ectosymbiotic and display only few anatomical modifications (Humes, 1986; Humes, 1998; Humes & Hendler, 1999; Kim, 2000; Ho & Kim, 2001). As pointed out above, *T. xenophiothricis* is the only one amongst them to show a particular colouration that mimics perfectly that of its host; this complex and uncommon mimicry could be indicative of high host specificity.

ACKNOWLEDGEMENTS

The authors thank Dr I. Eeckhaut (UMH), Dr D. Van den Spiegel (UMH) and Mr G. Seghers (ULB) for their valuable help during sampling and for technical assistance on Laing Island. We also thank Prof. M. Jangoux (ULB & UMH) for permitting work in his laboratories. Funding for this research was provided by an FNRS (Fonds National pour la Recherche Scientifique, Belgique) grant to G. Doignon and by an FRFC convention (contract number 2.4560.96F). Contribution of the Centre Interuniversitaire de Biologie Marine (CIBIM), Belgium.

REFERENCES

Barel, C.D.N. & P.G.N. Kramers (1977). A survey of the echinoderm associates of the north-east Atlantic area. *Zool. Verh., Leiden*, 156 : 1-159.

Bartsch, I. (1994). Siphonostomatoide copepoden auf Ophiacanthiden (Ophiuroidea). Beschreibung von drei *Cancerilla*-arten (Cancerillidae, Copepoda) aus der Antarktis. *Mitt. Hamb. Zool. Mus. Inst.,* 91 : 61-76.

Bartsch, I. (1996). Parasites of the Antarctic brittle star *Ophiacantha disjuncta* (Ophiacanthidae, Ophiuroidea). Redescription of the copepod *Lernaeosaccus ophiacanthae* Heegaard, 1951. *Mitt. Hamb. Zool. Mus. Inst.,* 93 : 63-72.

Boxshall, G.A. (1988). A review of the copepod endoparasites of brittle stars (Ophiuroidea). *Bull. Brit. Mus. Nat. Hist. (Zool.)*, 54 : 261-270.

Boxshall, G.A. & S. Ohtsuka (2001). Two new families of copepods (Copepoda : Siphonostomatoida) parasitic on echinoderms. *J. Crust. Biol.*, 21 : 96-105.

Britayev, T.A., G. Doignon & I. Eeckhaut (1999). Symbiotic polychaetes from Papua New Guinea associated with echinoderms, with the description of three new species. *Cah. Biol. Mar.*, 40 : 359-375.

Canu, E. (1892). Les Copépodes du Boulonnais. Morphologie, Embryologie, Taxonomie. *Trav. Lab. Zool. Mar. Wimereux-Ambleteuse*, 6 : 1-354, Pl. 1-30.

Dahms, H.-U. (1990). The first nauplius and the copepodite stages of *Thalestris longimana* Claus, 1863 (Copepoda, Harpacticoida, Thalestridae) and its bearing on the reconstruction of phylogenetic relationships. *Hydrobiologia*, 202 : 33-60.

Gooding, R.U. (1957). On some Copepoda from Plymouth, mainly associated with invertebrates, including three new species. *J. Mar. Biol. Ass. U.K.*, 36 : 195-221.

Gotto, V. (1993). *Commensal and parasitic copepods associated with marine invertebrates (and whales)*. In : Synopses of the British fauna (new series), Vol. 46. Kermack, Barnes & Crothers (Eds). Linnean Society of London & The Estuarine and Coastal Sc. Associated, Oegstgeest, The Netherlands : 1-264.

Ho, J.-S. & I.-H. Kim (2001). New species of *Doridicola* (Copepoda, Rhynchomolgidae) from Thailand, with a cladistic analysis of the genus. *J. Crust. Biol.*, 21 : 78-89.

Humes, A.G. (1982). A review of Copepoda associated with sea anemones and anemone-like forms (Cnidaria, Anthozoa). *Trans. Amer. Phil. Soc.*, 72 : 1-120.

Humes, A.G. (1986). Two new species of Copepoda associated with the basket star *Astroboa nuda* (Ophiuroidea) in the Moluccas. *Zool. Scripta*, 15 : 323-332.

Humes, A.G. (1990). Synopsis of lichomolgid copepods (Poecilostomatoida) associated with soft corals (Alcyonacea) in the tropical Indo-Pacific. *Zool. Verh., Leiden*, 266 : 1-201.

Humes, A.G. (1994). How many copepods? *Hydrobiologia*, 292/293 : 1-7.

Humes, A.G. (1998). Copepoda (Siphonostomatoida) associated with Ophiuroidea in Jamaica, Puerto Rico, and Barbados. *Zool. Verh., Leiden*, 323 : 365-382.

Humes, A.G. & G.A. Boxshall (1996). A revision of the lichomolgoid complex (Copepoda : Poecilostomatoida), with the recognition of six new families. *J. Nat. Hist.*, 30 : 175-227.

Humes, A.G. & G. Hendler (1999). Biology and taxonomy of species of *Ophiopsyllus* and *Pseudanthessius* (Copepoda) associated with brittle stars (Ophiuroidea) in Belize. *Bull. Mar. Sc.*, 65 : 699-713.

Humes, A.G. & J.H. Stock (1973). A Revision of the Family Lichomolgidae Kossmann, 1877, Cyclopoid Copepods mainly associated with Marine Invertebrates. *Smiths. Contr. Zool.*, 127 : 1-368.

Huys, R. & G.A. Boxshall (1991). *Copepod evolution*. The Ray Society, London (468 pp).

Jangoux, M. (1990). Diseases of Echinodermata. In : *Diseases of Marine Animals (Vol. 3)*. Kinne (Ed.). Biologischen Anstalt Helgoland, Hamburg : 439-567.

Kim, I.-H. (2000). Poecilostomatoid copepods from an intertidal mud flat in the Yellow Sea. *J. Nat. Hist.*, 34 : 367-432.

Williams, E.H. Jr & T.J. Wolfe-Waters (1990). An abnormal incidence of the commensal copepod, *Doridicola astrophyticus* Humes, associated with injury of its host, the basketstar, *Astrophyton muricatum* (Lamarck). *Crustaceana*, 59 : 302.

Belg. J. Zool., 134 (1) : 75-78 March 2004

Preliminary study of the scars borne by Gammaridae (Amphipoda, Crustacea)

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ABSTRACT. Many gammarid shrimps from the rivers Meuse and Viroin bear scars. A recent invader in the Meuse, *Dikerogammarus villosus*, is claimed to exhibit a strong predatory behaviour against other Gammaridae. Therefore we tested the hypothesis that scarred Gammaridae should be more numerous in the Meuse where *D. villosus* is dominant than in a tributary, the Viroin, where it is absent. On the contrary there were significantly more scarred individuals in the Viroin (51%) than in the Meuse (32%). The most exposed appendages were the most frequently injured, and multiple scars on a single individual were not distributed randomly. These results are discussed.

KEY WORDS : invasive species, *Dikerogammarus*, *Echinogammarus*, *Gammarus*.

INTRODUCTION

Dikerogammarus villosus (Sowinsky 1874) (Amphipoda, Gammaridae) originated from the Ponto-Caspian region, which has been claimed to be an "invasion donor hot spot" (Ricciardi & MacIsaac, 2000; Dick & Platvoet, 2001). The penetration of *D. villosus* into Western Europe was facilitated by the reopening of the Main-Danube Canal in 1992 (Tittizer, 1996 in Dick & Platvoet, 2001, Bij de Vaate et al., 2002). The mechanism of this invasion was most probably passive transport in ballast water (Tittizer et al., 2000). *D. villosus* was first recorded in the Belgian Meuse in 1998 (Vanden Bossche, 2001) and has now become dominant, but it has not yet penetrated into the Meuse's tributaries.

Cannibalistic and predatory behaviours are well known and described in Gammaridae (Dick, 1995, Dick et al., 1999) and in the case of *D. villosus* intraguild predation seems to be even more frequent. Whereas other Gammaridae are restricted in their attacks to the few minutes following the moult of their victims (when their exoskeleton is still soft), very hard mouthparts allow *D. villosus* to prey upon intermoult individuals (with hard exoskeleton) (Dick & Platvoet, 2000).

When injured, amphipods, as do other invertebrates, trigger activation of the prophenoloxidase cascade (Johansson & Soderhall, 1996). This nonspecific immunoreaction results in black scars, which are easy to locate. To our knowledge these scars have never been studied as witnesses of agonistic relationships in amphipod communities. Knowing the predatory reputation of *D. villosus*, we tested the hypothesis that scarred Gammaridae should be less numerous in the River Viroin (*D. villosus* absent, *Echinogammarus berilloni* (Catta, 1878) dominant) than in the River Meuse (*E. berilloni* present, *D. villosus* dominant and the most abundant interstitial macroinvertebrate in this ecosystem).

MATERIAL AND METHODS

Amphipods were sampled in the river Meuse at Montigny-sur-Meuse, France (UTM co-ordinates U31 0623356 5545159, altitude 110 m), and in one of its tributaries, the river Viroin at Mazée, Belgium (UTM co-ordinates U31 0620956 5550380, altitude 115 m), from October 2001 to May 2002.

Interstitial macroinvertebrates were collected with artificial substrates. These consist of calcareous gravel (5.2 ± 3.4 cm^3 : av ± st dev) packed in a nylon net. Macroinvertebrates can enter and exit freely through the 1 cm-mesh. The volume of an artificial substratum is 1 litre and contains an interstitial space of about 0.5 litre. The substrates remained in the water and were replaced by new ones every two weeks (each month during winter). Samples were preserved in alcohol with picric acid, which stops the immunoreaction. In this way we could distinguish the natural scars (black) from the injuries caused by our sampling manipulations (not coloured). About 450 individuals from each river were examined : they were identified with the following keys : Carausu et al. (1955), Schellenberg (1942), Pinkster (1973), Karaman & Pinkster (1977) and Pinkster (1993). Every scar was counted, and its location on the body and the appendages noted.

The gammarid species encountered in the Meuse are (in decreasing order of numbers) : *Dikerogammarus villosus* (Sowinsky, 1874), *Echinogammarus berilloni* (Catta, 1878) and *Gammarus pulex* (L., 1758). In the Viroin, we found *E. berilloni*, *G. pulex* and a few *Gammarus fossarum* (Koch, 1835).

During the sampling period (October 2001-May 2002) all the adult and pre-adult individuals (i.e. 467 from the Viroin and 442 from the Meuse) were scrutinised for scars. Two kinds of scars were considered : (a) those borne on the gills and (b) those borne elsewhere, mainly on the appendages but also on the body. A possible seasonal effect was tested by distributing our samples into

three periods : autumn 2001 (from October 27 until November 25), winter (from December 21 until March 15) and spring 2002 (from March 30 until May 24).

Unless otherwise stated the statistical analyses were performed with χ^2 contingency tests for independent samples; in case of 2x2 contingency tables, the correction for continuity was applied (SIEGEL & CASTELLAN, 1988).

RESULTS

The scars borne on the gills appear as black borders; sometimes a gill is pierced and the hole is black edged. These scars obviously cannot have been made by a predator but probably result from infectious micro-organisms. The results by river and species are figured in table 1. The following statistical comparisons were performed : (1) between rivers for the scars borne on the gills of all Gammaridae, (2) the same for *E. berilloni* alone, (3) the same for *Gammarus* spp., (4) between *E. berilloni* and *Gammarus* spp. in the Viroin, (5) between *D. villosus* and. *E. berilloni* in the Meuse and (6) between *D. villosus* and. *E. berilloni* + *G. pulex* in the Meuse. None of them showed any significant differences (χ^2 tests, $p>0.05$ in all cases). The numbers were, however, too small for testing a seasonal effect on the gill scars.

TABLE 1

Distribution between rivers and species of Gammaridae with scarred gills

River	Species	# individuals scrutinised	# individual scarred	% scarred
Viroin	*E. berilloni*	437	29	6.6
Viroin	*G. pulex**	30	2	6.7
Meuse	*E. berilloni*	92	5	5.4
Meuse	*D. villosus*	317	12	3.8
Meuse	*G. pulex*	27	1	3.7

* Including some *G. fossarum* (only in the Viroin)

The scars borne on the appendages and sometimes on the body that might be attributed to predator bites were not distributed randomly. As expected, the most exposed appendages were the most frequently injured. More than 47% of the scars were located on the antennae and almost 37% on the other appendages. Scars on the body (head, tergites) were less frequent. The percentages of injured parts are detailed on Figure 1 and the results by river and species are given in Table 2.

TABLE 2

Distribution of Gammaridae with scarred appendages between rivers and species

River	Species	# individuals scrutinised	# individual scarred	% scarred
Viroin	*E. berilloni*	437	205	46.9
Viroin	*G. pulex**	30	22	73.3
Meuse	*E. berilloni*	92	32	34.8
Meuse	*D. villosus*	317	91	28.7
Meuse	*G. pulex*	27	11	40.7

* Including some *G. fossarum* (only in the Viroin)

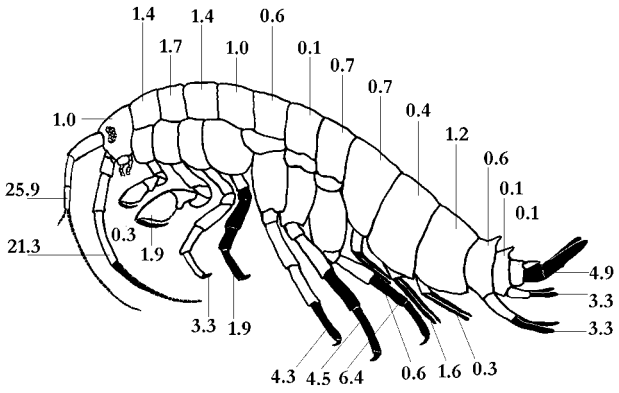

Fig. 1. – Distribution of scars on the body and the appendages : each number is the percentage occurrence of scars on the body and the appendages. The black parts are those that are most often lacking. Percentages on the coxae have been omitted for clarity.

There were very significantly more scarred individuals in the Viroin than in the Meuse (χ^2 tests, $p<0.001$ for all Gammaridae, $p<0.05$ for Gammarus spp. and $p<0.05$ for *E. berilloni* alone) : 50.7% of all the gammarid shrimps bore scars in the Viroin against 32.1% in the Meuse. There was no significant difference between species in the Meuse (χ^2 test, $p= 0.27$) but *Gammarus* spp. bore significantly more scars than *E. berilloni* in the Viroin (χ^2 test, $p<0.01$).

The results by river and season are figured in Table 3. The total catches followed a seasonal pattern (χ^2 test of homogeneity, $p<0.001$ in each river) and this pattern differed significantly between rivers (χ^2 test, $p<0.001$) with a drop in the winter period in the Meuse against a minimum in the spring in the Viroin.

TABLE 3

Distribution of Gammaridae with scarred appendages between rivers and seasons

River	Season	# individuals scrutinised	# individuals scarred	% scarred
Viroin	Autumn	166	51	30.7
Viroin	Winter	278	162	58.3
Viroin	Spring	23	14	60.9
Meuse	Autumn	204	68	33.3
Meuse	Winter	32	14	43.8
Meuse	Spring	200	52	26.0

The percentages of scarred individuals also followed a seasonal pattern in the Viroin (χ^2 test, $p=0.002$, with a lower percentage in the autumn) but the difference between seasons was not significant in the Meuse (χ^2 test, $p=0.23$)

The absolute frequency distributions of the number of scars borne by each individual are shown in Figure 2 and compared with random (Poisson's) distributions. In both rivers, the observed and theoretical frequency distributions differ very significantly (χ^2 test of goodness of fit, $p<0.001$). In both cases individuals without any scars were more numerous than expected in a random distribution, suggesting efficient hiding or escape from predators

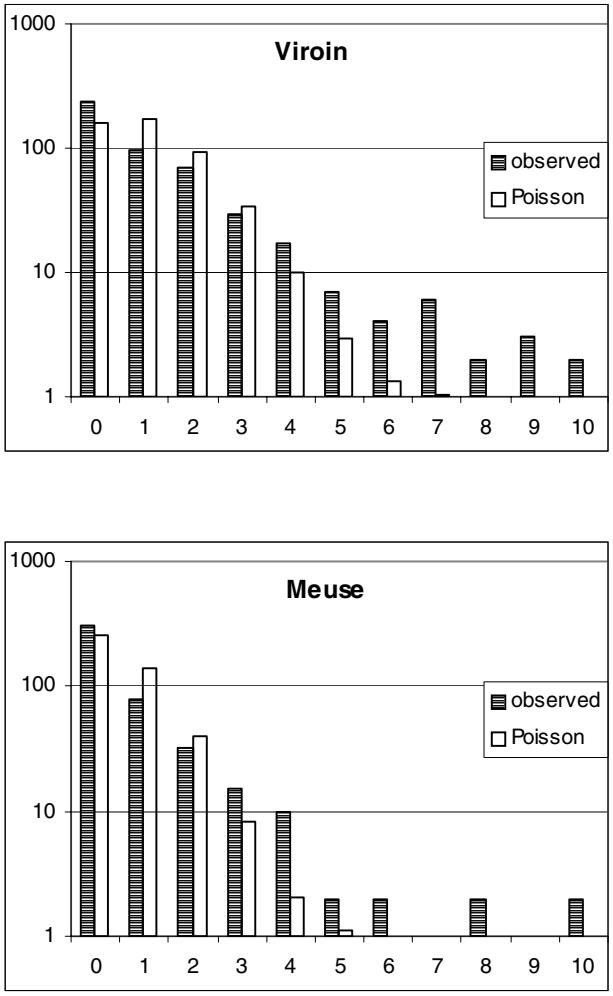

Fig. 2. – Observed and theoretical (Poisson) frequency distributions of scars among the Gammaridae (all species together) of the rivers Viroin and Meuse (semilog graphs of (x+1) transformed data).

following detection. Individuals bearing one or two scars were less numerous than expected and those with four or more scars were more numerous than expected.

Multiple scars on a single individual are not randomly spread on the body and the appendages : of 84 individuals bearing at least three scars, 36 individuals bore two to four scars on the antennae, 11 individuals bore two to five scars on successive tergites, seven individuals bore two to three scars on successive uropods of the same side, and six individuals bore two to five scars on successive pereiopods of the same side. Therefore 71% of those 84 individuals bore clumped scars.

DISCUSSION AND CONCLUSION

This study is part of a larger study on the population dynamics of Gammaridae. The scars that they bear, to our knowledge, have never been taken into consideration in the literature. These scars, however, could provide valuable clues for understanding some kinds of relationships occurring in river communities, as aggressive behaviours and failed attempts of predation.

The communities of invertebrate predators in the rivers Viroin and Meuse are not very different : they include leeches (Erpobdellidae and Glossiphoniidae), flatworms (Dugesiidae) and a variety of insect larvae : small numbers of damselflies (Calopterygidae and Lestidae) and beetles (Gyrinidae), and larger numbers of net building caddisflies (Hydropsychidae, Polycentropodidae and Rhyacophilidae) and snipeflies (Rhagionidae). There is, however, one recent invader, *Dikerogammarus villosus*, which is totally lacking in the Viroin and very abundant in the Meuse. From its known aggressive and predatory behaviour against other gammarids (Dick et al., 1999, Dick & Platvoet, 2000), more scarred shrimps might be expected in the Meuse but the opposite was observed. Of course it could be argued that *D. villosus* is such an efficient predator that it seldom lets its prey escape. On the other hand the other Gammaridae, which also exhibit agonistic behaviours but do not kill their prey unless it has moulted quite recently (Dick, 1995, Dick et al., 1999), could be the origin of the numerous scars observed in the Viroin. Obviously controlled experiments (Dick et al., 1999) should be carried out in order to appreciate to which extent intraspecific and interspecific aggressiveness can generate these scars.

Even if any of the invertebrate predators could be responsible for the isolated scars borne by the Gammaridae, none of them can account for the frequent clumped distribution of multiple scars. Fishes that forage on the bottom of the rivers are probably better candidates as predators that may explain these results. Among others, the bullhead, *Cottus gobio* Linnaeus, is common in both rivers, and one bite of such a young fish could produce clumped multiple wounds that were observed on individuals bearing at least three scars.

Why, finally, should the gammarids bear more scars in the river Viroin? This could be explained by different predator densities but also by differences in their efficiencies linked with visibility. The water of the Meuse is never transparent; its turbidity actually varies to a large extent with discharge, boat navigation and plankton development. On the contrary the water of the Viroin can be turbid after a rain shower, but from late spring to mid-autumn it is more often limpid. In the Meuse the visual perception of prey by predators is therefore always more or less hampered by the water turbidity whereas in the Viroin it can be fairly good at some periods. The gammarid catches dropped noticeably when the water of the Viroin became clear in April-May, whereas their activity was rising in the Meuse (Table 3). Either they were severely preyed upon or their spring activity was inhibited in the Viroin by the water transparency. This again should be tested experimentally.

AKNOWLEDGEMENTS

We want to thank Mihai Pascu, Pierre Verboonen and Eddy Terwinghe for their invaluable help and technical assistance.

REFERENCES

Bij de Vaate, K., K. Jazdzewski, H.A.M. Ketelaars, S. Gollash & G. Van der Velde (2002). Geographical patterns in

range extension of Ponto-Caspian macroinvertebrate species in Europe. *Can. J. Fish. Aquat. Sci.,* 59 : 1159-1174.

Carausu, S., E. Dobreanu & C. Manolache (1955). Crustacea, Vol. IV, Fasc. 4. Amphipoda Forme salmastre si de apa dulce. In : Editura academiei republicii populare romine. *Fauna republicii populare romine* : 51-70.

Dick, J.T.A. (1995). The cannibalistic behaviour of two *Gammarus* species (Crustacea : Amphipoda). *J. Zool., Lond.,* 236 : 697-706.

Dick, J.T.A., W.I. Montgomery & R.W. Elwood (1999). Intraguild predation may explain an amphipod replacement : evidence from laboratory populations. *J. Zool., Lond.,* 249 : 463-468.

Dick, J.T.A. & D. Platvoet (2000). Invading predatory crustacean *Dikerogammarus villosus* eliminates both native and exotic species. *Proc. R. Soc. Lond. B,* 267 : 977-983.

Dick, J.T.A. & D. Platvoet (2001). Predicting future aquatic invaders. The case of *Dikerogammarus villosus*. *Aq. Nuis. Spec.*, 4 (3) : 25-27.

Johansson, M.W. & K. Soderhall (1996). The prophenoloxidase activating system and associated proteins in invertebrates. *Prog. Mol. Subcell. Biol*., 15 : 46-66.

Karaman, G.S. & S. Pinkster (1977). Freshwater *Gammarus* species from Europe, North Africa and adjacent regions of Asia (Crustacea – Amphipoda). Part I. *Gammarus pulex*-group and related species. *Bijdr. Dierk.,* 47 : 1-97.

Pinkster, S. (1973). The *Echinogammarus berilloni*-group, a number of predominantly Iberian amphipod species (Crustacea). *Bijdr. Dierk.,* 43 : 1-38.

Pinkster, S. (1993). A revision of the genus *Echinogammarus* (Stebbing, 1899) with some notes on related genera (Crustacea, Amphipoda). *Mem. Mus. Stor. Nat. (II Serie) Sci. Vitta (Biologia),* 10 : 9-23

Ricciardi, A. & H.J. MacIsaac (2000). Recent mass invasion of the North Great Lakes by Ponto-Caspian species. *Trends Ecol. Evol.,* 15 : 62-65.

Schellenberg, A. (1942). *Flohkrebse oder Amphipoda*. Tierweld Deutschlands, 40 (252 pp).

Siegel, S & N.J. Castellan, Jr (1988). *Nonparametric statistics for the behavioural sciences* (2d edition). McGraw-Hill int. Ed. (399 pp).

Tittizer, T., Schöll, F., Banning, M., Haybach. A. & M. Schleuter (2000). Aquatische Neozoen im Makrozoobenthos der Binnenwasserstrassen Deutschlands. *Lauterbornia,* 39 : 1-72.

Vanden Bossche, J.-P. (2001). First records and fast spread of five new (1995-2000) alien species in the River Meuse in Belgium : *Hypania invalida, Corbicula fluminea, Hemimysis anomala, Dikerogammarus villosus* and *Crangonyx pseudogracilis. Bull. Inst. Roy. Sci. Nat. Belg., Biol.,* 72 (Suppl.) : 73-78.

Belg. J. Zool., 134 (1) : 79-84 March 2004

Caloric restriction, Ins/IGF-1 signalling and longevity in the nematode *Caenorhabditis elegans*

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ABSTRACT. Several mechanisms of life span extension in *C. elegans* have been described, including caloric restriction, reduced Ins/IGF-1 signalling, Clk mutation and germ line ablation. Here, we describe the effects of caloric restriction on metabolism and life span in *C. elegans* and examine whether Ins/IGF-1 signalling is involved in the life extension observed in calorically restricted worms. We show that life span extension in restricted worms is not caused by a reduced metabolic rate, but is accompanied by enhanced stress resistance. We further show that caloric restriction and Ins/IGF-1 have additive effects on life span extension and on the stress defense enzyme activities, and that caloric restriction acts independently of *daf-16*. Thus, caloric restriction extends life span by mechanisms distinct from those affected by the Ins/IGF-1-like pathway.

KEY WORDS : aging, longevity, metabolism, *C. elegans*, caloric restriction, Ins/IGF-1 signalling.

INTRODUCTION

During the last two decades, more than 40 genes have been described that, when mutated, increase the life span of *C. elegans* (Johnson et al., 2000). Mutations in *daf-2* and *age-1* induce inappropriate dauer formation, but larvae that bypass the dauer stage at lower temperature can develop into adults that are long-lived (Friedman & Johnson, 1988; Kenyon et al., 1993). The longevity, or Age, phenotype is suppressed by mutations in the downstream gene *daf-16* (Lin et al., 1997). These and several other genes act in a common signalling pathway (Gottlieb & Ruvkun, 1994; Dorman et al., 1995), which shows homology to the mammalian insulin and IGF-1 signalling pathways : *daf-2* was found to encode an insulin/IGF-1 receptor (Kimura et al., 1997), and *age-1* a catalytic subunit of phosphoinositide-3-OH kinase (Morris et al., 1996). The protein encoded by *daf-16* is a forkhead transcription factor (Ogg et al., 1997; Lin et al., 1997), which is inactive and resides in the cytoplasm when phosphorylated by the Ins/IGF-1 signal, and relocates to the nucleus and controls transcription when dephosphorylated (Henderson & Johnson, 2001; Lin et al., 2001). Longevity mutants with reduced activities of the Ins/IGF-1 pathway were recently discovered in *Drosophila* (Clancy et al., 2001) and mice (Coschigano et al., 2000), suggesting that this pathway for life span control has an ancient origin.

A nutritionally complete, but calorie restricted, diet can significantly extend life span in a wide variety of both vertebrate and invertebrate animals and presumably humans as well (Finch, 1990; Lane, 2000). The mechanism by which caloric restriction prolongs life span is still unknown, however. It has been suggested that caloric restriction induces up-regulation of a variety of somatic maintenance functions (Yu, 1994; Masoro, 1995; Dhahbi et al., 2001) suggesting adaptive regulation and thus the existence of a specific pathway specifying the anti-ageing action of caloric restriction. This is sensible since a genetic program specifying enhanced maintenance in periods of food shortage would offer an appreciable selective advantage. Despite numerous suggestions that the Ins/IGF-1 pathway in worms may mediate the nematode response to caloric restriction (Apfeld & Kenyon, 1999; Lane, 2000; Guarente & Kenyon, 2000), no critical tests have been done to test this hypothesis.

A contrasting and quite popular hypothesis assumes that caloric restriction acts by decreasing oxidative stress (Sohal & Weindruch, 1996). Considering that a decrease of metabolic activity would result in reduced fluxes of reactive oxygen species (ROS), produced as by-products of metabolism and believed to be proximal causes of the aging process, some have favored the idea that the beneficial effect of caloric restriction is associated with a hypometabolic state (Lakowski & Hekimi, 1998; Lee et al., 1999). Thus according to this view, life extension resulting from caloric restriction is under thermodynamic rather than direct genetic control.

We used axenic culture medium, diluted *E. coli* concentration in suspension cultures, and the *eat-2 (ad1113)* mutant to study caloric restriction and the interaction with the Ins/IGF-1 pathway. The *eat-2 (ad1113)* mutant has a defect in pharyngeal pumping, resulting in reduced food uptake. Axenic medium supports sustained growth of *C. elegans* in the absence of a bacterial food source. Worms raised in this medium show several symptoms of caloric restriction including slenderness, delayed maturation, substantially extended and reduced fecundity and extended life span, as well as several biochemical alterations shared with *eat-2* mutants and wild-type worms grown on a restrictive bacterial diet (Houthoofd et al., 2002a, b).

MATERIAL AND METHODS

Culture conditions

Monoxenic (containing one additional species) age synchronous cultures were established as follows. Eggs were prepared by hypochlorite treatment of gravid adults and allowed to hatch overnight in S buffer (Sulston & Hodgkin, 1988), and the resulting first stage larvae (L1) were inoculated onto freshly prepared cholesterol-supplemented nutrient agar (OXOID) plates containing a lawn of *E. coli* 9001 cells. Fourth stage larvae (L4) were transferred into Fernbach flasks containing 250 ml of aging medium, which was S buffer containing approx. $3x10^9$ bacterial cells/ml and 50 μM FUdR (to prevent reproduction). The bacteria were added as frozen beads containing equal volumes of pelleted bacteria and S buffer. The flasks were shaken in a temperature controlled (24 ˚C) gyratory shaker at 120 oscillations/min.

Axenic cultures were established by two consecutive cycles of hypochlorite treatment to achieve sterility. L1s were inoculated into axenic culture medium. Axenic culture medium contained 3% (w/v) soy-peptone and 3% (w/v) dry yeast extract and was sterilized under standard conditions. After cooling, hemoglobin stock solution was diluted 100 fold into the basal medium. Hemoglobin stock solution was prepared by dissolving 5 g hemoglobin in 100 ml 0.1 N KOH, and autoclaving for no longer than 10 min at 121 ˚C to reduce excessive hydrolysis. To improve synchronous development, heat-killed *E. coli* cells were added at $3x10^9$ cells/ml. As soon as the worms reached the fourth larval stage, they were washed with sterile S buffer and suspended into Fernbach flasks containing 250 ml axenic medium (without heat killed bacteria). FUdR was added at 50μM final concentration. Culture conditions were as described for the monoxenic cultures.

Metabolic analyses

An aliquot of a worm culture was harvested daily or every second day and cleaned using Percoll (Fabian & Johnson, 1994) and sucrose density gradients, as previously described (Braeckman et al., 2002). The respiration and heat output assays required live worms. Cleaned worm samples for other assays were frozen and stored at –75 ˚C. Consumption of dissolved oxygen by suspended worms was monitored using Clark electrodes, and heat output was measured by microcalorimetry (Braeckman et al., 2002). Oxygen consumption was performed using worms that were suspended in liquid, as required for polarographic respirometry (Clark electrodes), but heat measurements were made under both growth conditions. Even so, all worms were suspended in liquid for the sucrose and Percoll washes, and we cannot guarantee that the metabolic activities in culture and under the assay conditions were identical. This is a caveat to comparing metabolic activity of worms raised on plates or in suspension.

ATP levels were measured by using the well-established luciferin-luciferase assay adapted for use in a microtiter plate format as described previously (Braeckman et al., 2002). This assay is based on the reaction : luciferin + ATP + O_2 → oxyluciferin + AMP + pyrophosphate + CO_2 + light. The flash frozen nematode samples (100 μl) were taken from the –75 ˚C freezer and immediately submersed in a boiling water bath for 15 min to destroy ATPase activity and to allow diffusion of ATP out of the corpses. Dilutions were made using HPLC grade water (salts interfere with the assay) and the assay was performed according to the manufacturers' (Roche Diagnostics GmbH, Germany) instructions. This assay is several orders of magnitude more sensitive than colorimetric assays. We measured levels of approx. 1.4 mM ATP in young adult wild-type worms, approaching the range of values (2-10 mM) detected in other forms of life (Lehninger, 1981). Comparative assays using live worms that were boiled and sonicated to maximize ATP recovery indicated that our protocol underestimates true values by about 16%. We consider this mild underestimation fully acceptable in view of the convenience afforded by using stored worms.

We assessed total bioreduction capacity using XTT. Tetrazolium salts, especially MTT, are widely used for measuring the redox potential of cells as a parameter of viability (Johnson, 1995). This approach is based on the reduction of MTT, within active mitochondria of living cells, by succinate dehydrogenase to an insoluble blue formazan derivative. Tetrazolium salts can also be used to score dehydrogenase activity in cellular extracts. These methods commonly monitor phenazine methosulfate (PMS) -mediated reduction of tetrazolium by various NAD(P) dependent dehydrogenases (Altman, 1972; Richardson et al., 1986). Unlike most previous tetrazolium compounds, XTT [(2,3-bis-(2-methoxy-4 nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide] is reduced to a water-soluble formazan, allowing direct monitoring of its appearance. We found no activity when these tetrazolium salts were added to worm homogenates (made 1% in CHAPS) in the absence of PMS. However, formazan formation proceeded upon addition of reduced nicotinamide cofactors. Conversely, reduced nicotinamide cofactors were unable to reduce XTT directly in the absence of appropriate electron acceptors such as phenazine methosulfate. Thus worm homogenate reduces XTT in the presence of NAD(P)H, and the amount of formazan produced increases linearly with the amount of tissue extract added (Braeckman et al., 2002).

We found that exogenous SOD lowered XTT reduction by 50% suggesting that this amount is due to superoxide, and we infer that the activity that is not suppressible by SOD is contributed by unknown NAD(P)H-dependent reductase(s). This assay can be used to monitor changes in XTT bioreduction capacity that occur as a function of metabolic alterations, including those that accompany aging. Thus, although the precise biochemical targets of the assay are as yet obscure, we consider the XTT reduction capacity an excellent biomarker of aging.

SOD activity was measured by an assay based on the inhibition of superoxide-induced lucigenin chemiluminescence by SOD (Corbisier et al., 1987). Aliquots of 6.7 μl were taken from a sample dilution series and added in duplicate to the wells of a microtiter plate. Next, 20 μl aliquots of xanthine oxidase reagent (xanthine oxidase diluted in double distilled water such that the blank reaction containing 6.7 μl water, 20 μl XO dilution and 174 μl

reaction mixture yielded approx. 1.2 x 10^5 counts/s) and 174 µl of reaction mixture (5.2 ml 0.1 M glycine, 1 mM EDTA, adjusted to pH 9.0 with NaOH, 10 ml 0.108 mM xanthine, 2.1 ml 1 mM lucigenin, 1.2 ml water for a total of 18.5 ml) were added quickly by using a multichannel pipette. Luminescence was measured for 0.1 s during the time span required for 25 consecutive plate measurements at 25 ˚C using the Victor^2 Multilabel Counter. One unit of SOD activity is defined as the amount of SOD able to reduce the luminescence intensity by 50%. The homogenate fraction (dilution) reducing luminescence by 50% was derived mathematically from plots of the luminescence intensities measured as a function of the homogenate fraction. The sensitivity of this assay is superior to the standard cytochrome c assay (Vanfleteren, 1993; Vanfleteren & De Vreese, 1995) and the numerical values of SOD activity are not comparable.

Catalase activity was assayed at 25 ˚C according to the method of Aebi (1984), adapted for use in microtiter plate format. Briefly 6.9 µl sample volumes were added to the wells of a 96-well flat bottom UV transparant microtiter plate (UV-star, Greiner). The reaction was started by adding 200 µl substrate (11.4 mM hydrogen peroxide in 50 mM Na_2HPO_4 : KH_2PO_4 (Sørensen) buffer, pH 7.0) using a multichannel micropipette. The decrease in absorbance was monitored at 240 nm (Spectramax 190, Molecular Devices) for 25 reads (12 s interval, total measuring time : 4 min, 17s). The amount of peroxide decomposed was calculated using a molar coefficient of $\varepsilon_{240nm, 1cm}$=39.4. The enzyme activity decomposing 1µmole of hydrogen peroxide per min equals 1 unit catalase activity.

The experimental data were scaled to protein content to correct for differences in biomass. The metabolic measurements were repeated several times to reduce assay variation. The results were obtained from three or four independent source populations to control for environmental variation.

RESULTS AND DISCUSSION

Our primary interest was to determine if caloric restriction causes a reduction of the metabolic rate, as is often assumed. We therefore measured the oxygen consumption and the heat output of calorically restricted worms (Fig. 1a and 1b). We found that worms that undergo caloric restriction by three different means had significantly higher metabolic rates, suggesting that the life span extension of restricted worms is not caused by reduced

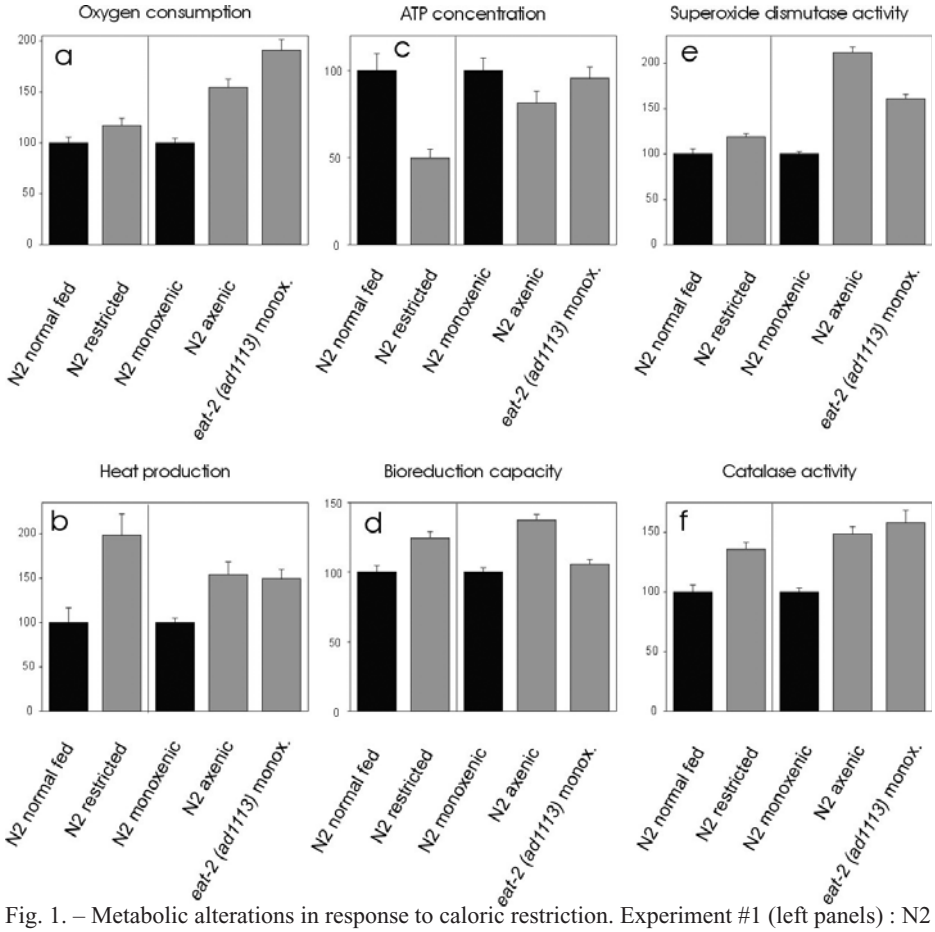

Fig. 1. – Metabolic alterations in response to caloric restriction. Experiment #1 (left panels) : N2 normal fed : two-day-old adults of N2, grown in Fernbach flasks. Average of cultures supplied with 15, 6 and 2.4 x 10^9 *E. coli* cells/ml; N2 restricted : two-day-old adults of N2, grown in Fernbach flasks. Average of cultures supplied with 96, 38, 15 and 6 x 10^7 *E. coli* cells/ml. Average of four replicates. Experiment #2 (right panels) : N2 and *eat-2 (ad1113)* monoxenic : worms grown in Fernbach flasks containing 3x10^9 *E. coli* cells/ml S buffer. N2 axenic : N2 grown in axenic medium. Average over the first ten days of the adult life span of three-five replicates. All parameter values relative (%) to the normal fed wild type controls. All strains were grown at 24˚C.

metabolic activity. We reasoned that partial starvation may enhance ATP and NADPH consumption activity for *de novo* synthesis of biomolecules that are otherwise supplied with food. We therefore measured the ATP concentration in the worms and found less ATP in calorically restricted worms (Fig. 1c). We also measured the worms' ability to reduce XTT, since we have discovered that this capacity is strongly enhanced in non-feeding dauers (Houthoofd et al., 2002c), which can survive several times the normal life span (Klass & Hirsh, 1976). We found elevated XTT reduction capacity in worms grown in liquid medium, supplemented with less *E. coli* and in worms grown in axenic medium (Fig. 1d). However, the *eat-2 (ad1113)* mutant did not show elevated XTT reduction capacity, but the age dependent decline was slower (not shown), resulting in higher reduction capacity at older age. Perhaps the increase in bioreduction capacity partly serves the *de novo* synthesis of biomolecules. The observed metabolic effects were not caused by reduced progeny production, since a mutant lacking a germ line did show similar effects (not shown).

If a reduced metabolic rate is not the reason for the longevity phenotype of restricted worms, then one could assume that increased defense against oxidative stress could be the primary cause. We therefore measured the catalase and SOD activity of restricted worms. We found that the activity of both enzymes was increased in response to caloric restriction (Fig. 1e and 1f). We therefore conclude that caloric restriction increases the life span of *C. elegans* by increasing the ROS defense.

We further asked what pathway could regulate the increase in life span of calorically restricted worms. The Ins/IGF-1 pathway is an obvious candidate, since it is known that it controls life span in *C. elegans*. This phosphorylation cascade pathway tranduces a signal from the DAF-2 receptor to AKT proteins. AKT proteins regulate the localization of the transcription factor DAF-16 (Henderson & Johnson; 2001). Nuclear localization occurs in the absence of signalling due to lack of ligand binding or to mutation in one of the proteins in the pathway, and results in increased adult life span. To answer our question, we grew Ins/IGF-1 mutants under caloric restriction circumstances and determined the life span, stress defense and metabolic alterations.

We found that the mean life span of wild type was increased (153%; Fig. 2). Moreover, *daf-2(e1370)* results in life extension of 274% when the worms are grown in axenic medium, whereas this mutant extended life span by 69% relative to wild-type on plate cultures. Caloric restriction and reduced Ins/IGF-1 signalling thus lengthen the life span of *C. elegans* in a synergistic way, consistent with independence of caloric restriction from the Ins/IGF-1 pathway. This dramatic increase in longevity of *daf-2* under caloric restriction is the longest mean (90.9 days) and maximum (136 days) life span yet reported for *C. elegans* and argues dramatically for independence of the caloric restriction and *daf-2* pathways. We also investigated whether *daf-16* mutation, which has been reported to suppress the life extension of Ins/IGF-1 mutants to that of wild type (Kenyon et al., 1993; Gottlieb & Ruvkun, 1994), can suppress caloric restriction-induced life extension. *daf-16* mutants showed increased life expectancy averaging 152% in axenic culture, relative to *E. coli* plate cultures. These results indicate that life span extension caused by growth in axenic medium does not require *daf-16.* Since *daf-2* specified life span extension is *daf-16* dependent, caloric restriction and the Ins/IGF-1 pathway must be distinct.

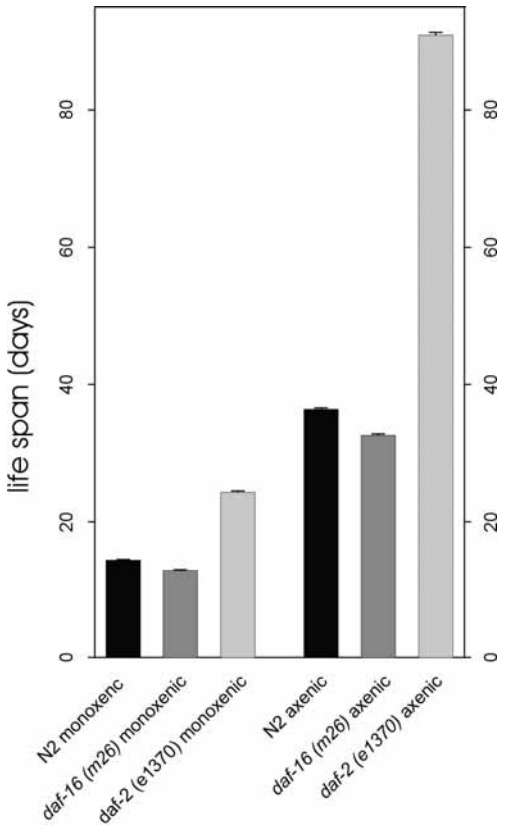

Fig. 2. – Life span in response to caloric restriction and Ins/IGF-1 signalling. Average life span of N2, *daf-16 (m26)* and *daf-2 (e1370)* at 24˚C on monoxenic agar plates and in axenic medium.

Both SOD and catalase activities are elevated in *daf-2* and reduced in *daf-16* in bacterial culture and relative to wild-type (Fig. 3a and b). *daf-16* largely suppresses the *daf-2* SOD phenotype, consistent with other evidence that *daf-16* controls expression of SOD (Honda & Honda, 1999). However, growth in axenic medium causes elevation of SOD and catalase in all strains, suggesting that caloric restriction defines a separate pathway.

The DAF-16 protein is a transcription factor that becomes localized in the nucleus in response to upstream signals as well as to a series of stressors including heat, oxidative stress and starvation (Henderson & Johnson, 2001). If life span extension by caloric restriction is mediated by the Ins/IGF-1 pathway, then DAF-16 should respond to the stimulus by moving to the nucleus. A DAF-16 : :GFP construct, grown in axenic medium, shows no nuclear localization, however (not shown). This is consistent with the cytosolic localization of DAF-16 : :GFP in an *eat-2* mutant (Henderson & Johnson, 2001), and argues against a role for the Ins/IGF-1 pathway in mediating life extension by caloric restriction.

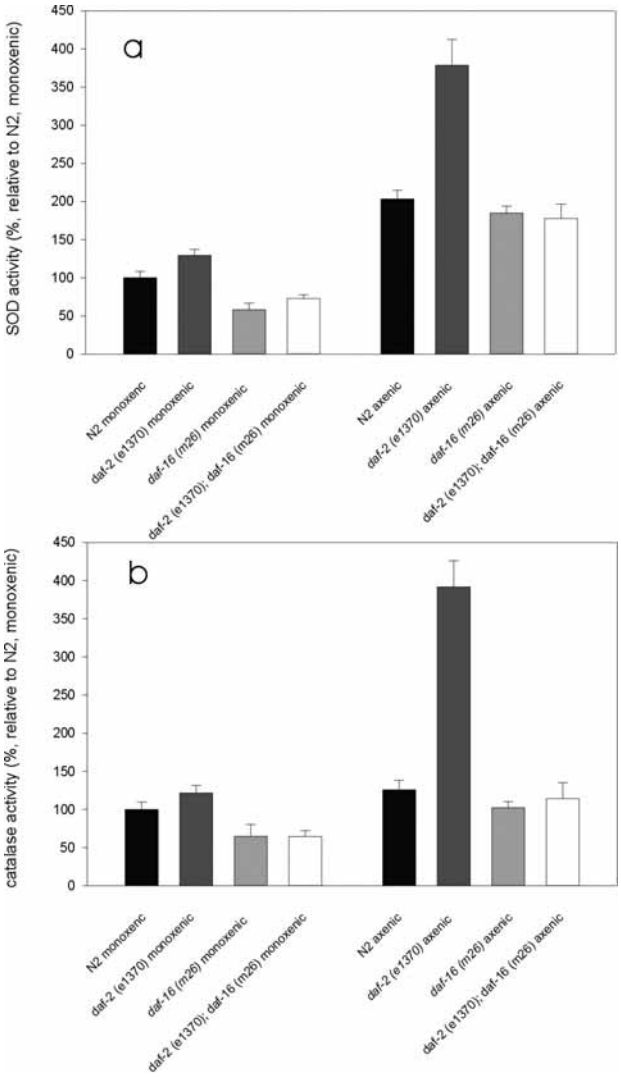

Fig. 3. – SOD and catalase activity in response to caloric restriction and Ins/IGF-1 signalling. Average activity over the total life span of N2, *daf-16 (m26)*, *daf-2 (e1370)* and *daf-2 (e1370) ; daf-16 (m26)* at 24˚C. Monoxenic worms were grown in Fernbach flasks supplied with at least $3x10^9$ *E. coli* cells/ml. Axenic worms were grown in Fernbach flasks with axenic medium.

In conclusion, we found that caloric restriction and deficiencies in the Ins/IGF-1 pathway caused additive effects on life span and SOD and catalase activity. Furthermore, extension of life span and increased activity of SOD and catalase that are caused by caloric restriction, are also seen in *daf-16* mutants, suggesting that caloric restriction does not need intact *daf-16* activity. These conclusions are consistent with DAF-16 residing outside the nucleus in calorically restricted *eat* mutants and wild-type worms raised in axenic culture.

ACKNOWLEDGEMENTS

K.H. and B.B. are postdoctoral fellows with the Fund for Scientific Research - Flanders, Belgium. All strains used in this study were provided by the *Caenorhabditis* Genetics Centre, which is funded by the National Institutes of Health, National Centre for Research Resources. This research was supported by grants form Ghent University, the Fund for Scientific Research-Flanders, and the European Commission.

REFERENCES

Aebi, H. (1984). Catalase *in vitro*. *Methods Enzymol*., 105 : 121-126.

Altman, F.P. (1972). Quantitative dehydrogenase histochemistry with special reference to the pentose shunt dehydrogenases. *Cytochemistry,* 4 : 225-273.

Apfeld, J. & C. Kenyon (1999). Regulation of lifespan by sensory perception in *Caenorhabditis elegans*. *Nature,* 402 : 804-9.

Braeckman, B.P., K. Houthoofd, A. De Vreese & J.R. Vanfleteren (2002). Assaying metabolic activity in ageing *Caenorhabditis elegans*. *Mech. Ageing Dev.,* 123 : 105-19.

Clancy, D.J. *et al.* (2001).Extension of life-span by loss of CHICO, a *Drosophila* insulin receptor substrate protein. *Science,* 292 : 104-6.

Corbisier, P., A. Houbion & J. Remacle (1987). A new technique for highly sensitive detection of superoxide-dismutase activity by chemiluminescence. *Anal. Biochem.,* 164 : 240-247.

Coschigano, K.T., D. Clemmons, L.L. Bellush & J.J. Kopchick (2000). Assessment of growth parameters and life span of GHR/BP gene-disrupted mice. *Endocrinology,* 141 : 2608-13.

Dhahbi, J.M. *et al.* (2001).Caloric restriction alters the feeding response of key metabolic enzyme genes. *Mech. Ageing Dev.,* 122 : 1033-48.

Dorman, J.B., B. Albinder, T. Shroyer & C. Kenyon (1995). The *age-1* and *daf-2* genes function in a common pathway to control the lifespan of *Caenorhabditis elegans*. *Genetics,* 141 : 1399-406.

Fabian, T.J. & T.E. Johnson (1994). Production of age-synchronous mass cultures of *Caenorhabditis elegans*. *J. Gerontol. Biol. Sci.,* 49B : 145-156.

Finch, C.E. (1990). *Longevity, Senescence and the Genome*. University of Chicago Press, Chicago.

Friedman, D.B. & T.E. Johnson (1988). A mutation in the age-1 gene in *Caenorhabditis elegans* lengthens life and reduces hermaphrodite fertility. *Genetics,* 118 : 75-86.

Gottlieb, S. & G. Ruvkun (1994). *daf-2*, *daf-16* and *daf-23* : genetically interacting genes controlling Dauer formation in *Caenorhabditis elegans*. *Genetics,* 137 : 107-20.

Guarente, L. & C. Kenyon (2000). Genetic pathways that regulate ageing in model organisms. *Nature,* 408 : 255-62.

Henderson, S.T. & T.E. Johnson (2001). *daf-16* integrates developmental and environmental inputs to mediate aging in the nematode *Caenorhabditis elegans*. *Curr. Biol.,* 11 : 1975-1980.

Honda, Y. & S. Honda (1999). The *daf-2* gene network for longevity regulates oxidative stress resistance and Mn-superoxide dismutase gene expression in *Caenorhabditis elegans*. *FASEB J,* 13 : 1385-93.

Houthoofd, K., B.P. Braeckman, I. Lenaerts, K. Brys, A. De Vreese, S. Van Eygen & J.R. Vanfleteren (2002a). No reduction of metabolic rate in food restricted *C. elegans*. *Exp. Gerontol.,* 37 : 1357-1367.

Houthoofd, K., B.P. Braeckman, I. Lenaerts, K. Brys, A. De Vreese, S. Van Eygen & J.R. Vanfleteren (2002b). Axenic growth up-regulates mass-specific metabolic rate, stress resistance, and extends life span in *C. elegans*. *Exp. Gerontol.,* 37 : 1369-1376.

Houthoofd, K., B.P. Braeckman, I. Lenaerts, K. Brys, A. De Vreese, S. Van Eygen & J.R. Vanfleteren (2002c). Ageing is reversed, and metabolism is reset to young levels in recovering dauer larvae of *C. elegans*. *Exp. Gerontol.,* 37 : 1015-1021.

Johnson, J.E. (1995). Methods for studying cell death and viability in primary neuronal cultures. *Methods Cell. Biol.,* 46 : 243-276.

Johnson, T.E., J. Cypser, E. De Castro, S. DE Castro, S. Henderson, S. Murakami, B. Rikke, P. Tedesco & C. Link (2000). Gerontogenes mediate health and longevity in nematodes through increasing resistance to environmental toxins & stressors. *Exp. Gerontol.,* 35 : 687-694.

Kenyon, C., J. Chang, E. Gensch, A. Rudner & R.A. Tabtiang (1993). *C. elegans* mutant that lives twice as long as wild type. *Nature,* 366 : 461-464.

Kimura, K.D., H.A. Tissenbaum, Y. Liu & G. Ruvkun (1997). *daf-2*, an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans*. *Science,* 277 : 942-946.

Klass, M. & D. Hirsh (1976). Non-ageing developmental variant of *Caenorhabditis elegans*. *Nature,* 260 : 523-525.

Lakowski, B. & S. Hekimi (1998). The genetics of caloric restriction in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA,* 95 : 13091-13096.

Lane, M.A. (2000).Metabolic Mechanisms of Longevity : Caloric Restriction in Mammals and Longevity Mutations in *Caenorhabditis Elegans*; a Common Pathway? *J. Am. Aging Assoc.,* 23 : 1-7.

Lee, C.K., R.G. Klopp, R. Weindruch & T.A. Prolla (1999). Gene expression profile of aging and its retardation by caloric restriction. *Science,* 285 : 1390-1393.

Lehninger, A.L. (1981). *Biochemistry.* Worth Publishers, New York.

Lin, K., J.B. Dorman, A. Rodan & C. Kenyon (1997). *daf-16* : An HNF-3/forkhead family member that can function to double the life-span of *Caenorhabditis elegans*. *Science,* 278 : 1319-1322.

Lin, K., H. Hsin, N. Libina & C. Kenyon (2001). Regulation of the Caenorhabditis elegans longevity protein DAF-16 by insulin/IGF-1 and germline signalling. *Nat. Genet.,* 28 : 139-45.

Masoro, E.J. (1995). Dietary restricition. *Exp. Gerontol.,* 30 : 291-298.

Morris, J.Z., H.A. Tissenbaum & G. Ruvkun (1996). A phosphatidylinositol-3-OH kinase family member regulating longevity and diapause in Caenorhabditis elegans. *Nature,* 382 : 536-539.

Ogg, S., S. Paradis, S. Gottlieb, G.I. Patterson, L. Lee, H.A. Tissenbaum & G. Ruvkun (1997). The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in *C. elegans*. *Nature,* 389 : 994-9.

Richardson, B.J., P.R. Baverstock & M. Adams (1986). *Allozyme Electrophoresis A Handbook for Animal Systematics and Population Studies*. Academic Press, Sydney.

Sohal, R.S. & R. Weindruch (1996). Oxidative stress, caloric restriction, and aging. *Science,* 273 : 59-63.

Sulston, J. & J. Hodgkin (1988). Methods. In : *The Nematode Caenorhabditis elegans.* WOOD, WB. (Ed.). Cold Spring Harbor Laboratory, Plainview, New York : 587-602.

Vanfleteren, J.R. (1993). Oxidative stress and ageing in *Caenorhabditis elegans*. *Biochem. J.,* 292 : 605-608.

Vanfleteren, J.R. & A. De Vreese (1995). The gerontogenes age-1 and daf-2 determine metabolic rate potential in aging *Caenorhabditis elegans*. *FASEB J.,* 9 : 1355-61.

Yu, B.P. (1994). How diet influences the aging process of the rat. *Proc. soc. Exp. Biol. Med.,* 205 : 97-105.

APPENDIX : LIST OF ABBREVIATIONS

Ins :	Insulin
IGF-1 :	Insulin-like-growth-factor-1
ROS :	reactive oxygen species
PMS :	phenazine methosulfate
SOD :	superoxide dismutase
Note :	*daf-2* : gene, DAF-2 : gene product, Daf : phenotype (abnormal dauer formation)

Belg. J. Zool., 134 (1) : 85-88 March 2004

Local population dynamics of two co-existing birch aphid species : competition or intrinsic cycles of abundance ?

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ABSTRACT. Populations of four aphid species coexisting on their *Betula pendula* host-plant were followed in Brussels for two years. The population dynamics of *Euceraphis betulae* and *Symydobius oblongus* exhibited consistent patterns in 2001 and 2002, but *Callipterinella tuberculata* and *Betulaphis brevipilosa* populations exploded in 2001 and in 2002, respectively. When *C. tuberculata* was abundant, the population of *B. brevipilosa* was low. The reverse was true in 2002 as *C. tuberculata* became abundant only when the *B. brevipilosa* population declined, leading us to suspect competition between the two species. However, instead of avoiding each other, they were positively associated on shoots, probably as a result of aggregation at suitable feeding sites. Colonies of both species were similar or larger in mixed aggregates than in monospecific ones. Moreover, the colony size of each species in mixed aggregates was independent from that of the other species. Since there is no direct evidence of competition between those two species, the alternate changes in abundance could rather result from the intraspecific properties of aphid life cycles that self-induce consecutive years of high and low population growth in relation to fluctuations in the environmental conditions. These two co-existing species thus deserve further close study to elucidate their relationships.

KEY WORDS : aphids, *Betula pendula*, competition, population dynamics.

INTRODUCTION

The dynamics of aphid populations are complex and often unresolved phenomena, involving numerous regulating factors such as natural enemies, weather conditions, host-plant quality and defensive responses, complex polymorphic life cycles, as well as intra- and interspecific competition (Dixon, 1977). Among these factors, interspecific competition has been proved to be a very widespread phenomenon in guilds of herbivorous insects (for a review, see Denno et al., 1995).

Studies have widely reported the coexistence of several aphid species on the same host-plant, as well as niche differentiation between co-occurring species (Dixon, 1992). However, no clear-cut picture emerges concerning their interactions despite a large and often controversial body of literature that evidenced either no competition (e.g. Lawton & Strong, 1981; Hajek & Dahlsten, 1986) or direct (e.g. Bergeson & Messina, 1997; Gianoli, 2000) as well as indirect (e.g. Petersen & Sandstrom, 2001; Messina et al., 2002) competition between co-occurring aphid species.

A guild of at least fourteen aphid species relies more or less exclusively on birch trees (Atkinson, 1992), but little is known about their interactions. Hajek & Dahlsten (1986) described the important association of three aphid species coexisting on *Betula pendula* Roth trees. They hypothesized from the co-occurrence of the three species and from the high number of unoccupied leaves that interspecific competition was unlikely, although their niches largely overlapped.

We report here, in a native association in Belgium, the preliminary study of four aphid species co-occurring on *B. pendula* Roth. During two successive years, we made regular surveys of one tree characterized by high densities of aphids, where we hypothesized that competition phenomena were more likely to be detected. This 2-year study describes, with high resolution, the population dynamics of those aphid species, and tentatively identifies which mechanisms best account for changes in aphid abundance. Quite understandably, such a detailed approach is time-consuming and therefore precludes multiple replications in space and time. Nevertheless, such intensive study (as opposed to the more extensive sampling of classical ecology studies) is increasingly acknowledged as a good way to highlight the dynamics of a local population as well as its underlying mechanisms.

MATERIAL AND METHODS

Aphid populations were investigated in Brussels, Belgium, during 2001 and 2002. We counted aphids on all the shoots of a selected branch of a birch tree, *Betula pendula* Roth, growing as an ornamental on the university campus. Observations began from bud burst (early May in 2001 and early April in 2002) and repeated until almost complete fall of the leaves (late September-early October in both years). We counted the number and species of

aphids occurring on each shoot approximately every two weeks.

Four aphid species belonging to the Myzocallidinae sub-family and Calaphidini tribe were recognized on the tree in both years : *Symydobius oblongus* van Heyden (1837), *Euceraphis betulae* Koch (1855), *Betulaphis brevipilosa* Börner (1940) and *Calliperinella tuberculata* van Heyden (1837). They all are autoecious species restricted to birch trees (Atkinson 1992), and are easily distinguished in the field on the basis of size and colour differences. *S. oblongus* feeds primarily on the twigs of the present or preceding year, while the three other species feed mainly on leaves (personal observation). Very careful and gentle observation was required due to the great mobility of *C. tuberculata* and *E. betulae* and to the fact that all adults of the latter species are alates and fly readily when disturbed.

RESULTS

Population Dynamics

In both 2001 and 2002 the four aphid species co-occurred on the birch branch but the population dynamics of the guild differed greatly between the two years, as shown in Fig. 1a-b. *S. oblongus* was the least common species in both years though tended by *Lasius niger* L. ants throughout the season. Its demography was similar in 2001 and 2002, but it disappeared sooner in 2002. Most probably, the heavy rains of the beginning of July 2002 washed off this exposed large-sized aphid from the twigs. *E. betulae* also showed consistency in its demography in both years with a maximum abundance in May and extinction in mid-July.

On the other hand, the population dynamics of *C. tuberculata* and *B. brevipilosa* dramatically changed over successive years. *C. tuberculata* was very abundant in 2001 (Fig. 1a), with a drastic decline at the beginning of July followed by a smaller second peak of abundance in late August. *B. brevipilosa* was uncommon in 2001, almost rare, reaching its maximum population when *C. tuberculata* began its summer decline, and disappearing from the tree in the first days of August. In the following year the situation was reversed (Fig. 1b) : *B. brevipilosa* numerically dominated the aphid population from the end of May till the end of June 2002, and *C. tuberculata* was scarce until *B. brevipilosa* population began to collapse in mid-June.

Thus the population dynamics of both species in this local aphid community greatly differed from one year to the next. As population dynamics of aphids are quite complex phenomena (Dixon, 1977, 1992), we refined our study of *C. tuberculata* and *B. brevipilosa* populations in search of evidence that could allow a better understanding of the relationship between the two species. The following analyses took into account only mid-June data because the two species were simultaneously present in sufficient numbers at this time. Moreover, *C. tuberculata* was ant-tended in July and August which could dramatically alter its interactions with *B. brevipilosa* (this latter species being neither preyed on nor tended by *L. niger*, personal observation).

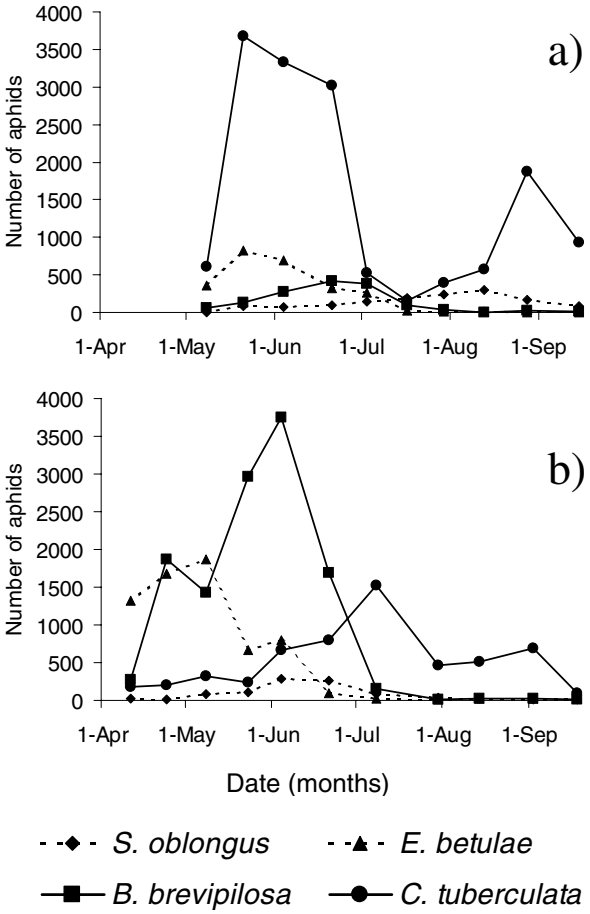

Fig. 1. – Population dynamics of four aphid species coexisting on *Betula pendula*, for a) the year 2001, and b) the year 2002.

Co-occurrence on shoots

We never noted avoidance or agonistic behaviour between the two species in either year. The number of birch shoots occupied by monospecific colonies of *C. tuberculata*, monospecific colonies of *B. brevipilosa*, or mixed colonies in both years are presented in Table 1. In 2001 and 2002 we found that the two species exhibited a positive association on shoots : they were significantly

TABLE 1

Occurrence of *C. tuberculata* and *B. brevipilosa* on birch shoots. For each year, the number of shoots occupied on the 21st June by monospecific colonies, mixed colonies or none, were compared using Fisher exact test.

		C. tuberculata		Fisher exact test
		Absent	**Present**	
B. brevipilosa	***2001***			
	Absent	25	22	p = 0.0002
	Present	5	33	
	2002			
	Absent	27	9	p = 0.0115
	Present	30	32	

more often found in mixed colonies than expected from random distribution (Fisher exact test : p=0.0002 and p=0.0115 in 2001 and 2002, respectively). A constrained crowding of both species due to lack of space was unlikely, since 29.4% and 27.5% of the shoots (still growing in June) remained unoccupied in 2001 and 2002, respectively.

Size of colonies in monospecific and mixed aggregates

For the two species of aphids in both 2001 and 2002, we compared the average number of individuals of a given species belonging either to a monospecific colony or an aggregate of both species. *C. tuberculata* in 2001 (Fig. 2a), as well as *B. brevipilosa* in 2002 (Fig. 2b) were more numerous in mixed aggregates than in monospecific ones (Welch corrected t-test : $t_{52=}3.169$, $p < 0.005$ for *C. tuberculata*; Mann-Whitney U test : U=379.5, $p < 0.0005$ for *B. brevipilosa*). However, we found no significant differences between population size in mixed and pure aggregates in 2002 for *C. tuberculata* and in 2001 for *B. brevipilosa*, when they were respectively less abundant (Welch corrected t-test : $t_{8=}1.330$, NS for *C. tuberculata*; Mann-Whitney U test : U=47.5, NS for *B. brevipilosa*).

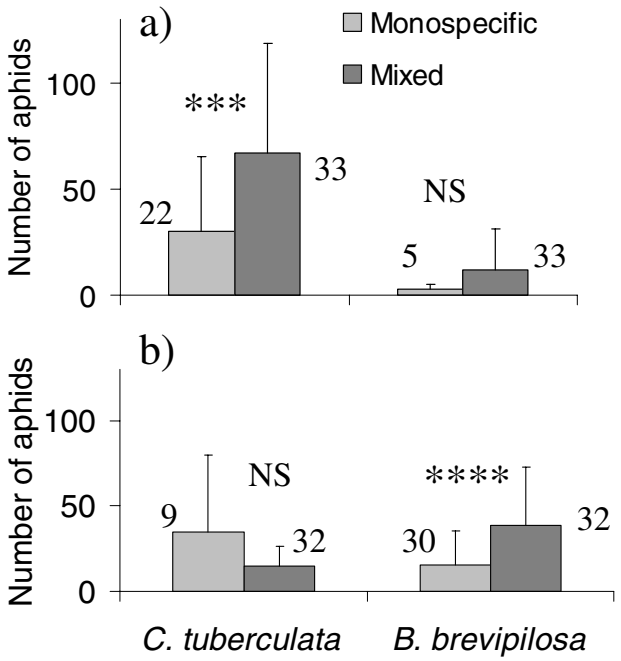

Fig. 2. – Number of individuals (mean ± SD) of *Callipterinella tuberculata* and *Betulaphis brevipilosa* found either in monospecific (light grey) or mixed (dark grey) aggregates on birch shoots in a) the year 2001, and b) the year 2002. Numbers indicate sample size. Statistical comparisons were made using Mann-Whitney test or Welch corrected t-test (*** : $p < 0.005$; **** : $p < 0.0001$).

In mixed aggregates, we failed to detect any correlation between the number of individuals belonging to one species and the number of individuals of the other species ($R^2=0.0054$, n=33, NS and $R^2=0.0008$, n=32, NS in 2001 and 2002 respectively, Fig. 3).

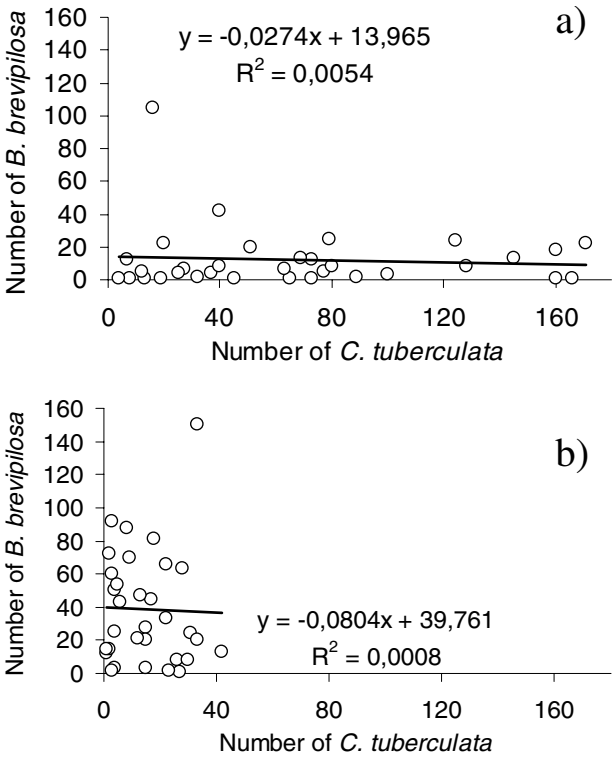

Fig. 3. – Relationship between the number of *Callipterinella tuberculata* and the number of *Betulaphis brevipilosa* in mixed aggregates in a) the year 2001 (n=33), and b) the year 2002 (n=32).

DISCUSSION

Here we showed that among four aphid species co-occurring on *B. pendula*, the local population dynamics of *B. brevipilosa* and *C. tuberculata* could vary greatly over successive years. But what do these variations of abundance actually reflect? The apparent inverse relation in the abundance of those two aphid species at this site might suggest the existence of interspecific competition.

At the level of the aphid colonies, we found *B. brevipilosa* and *C. tuberculata* positively associated on birch shoots. This observation is in agreement with the work of Hajek & Dahlsten (1986), in which the niche of *B. brevipilosa* overlapped widely with that of another *Callipterinella* species (*C. calliptera* Hartig), although a large number of leaves remained unoccupied by aphids. However, this might simply reflect the higher quality of the locations of mixed aggregates compared with those of monospecific ones. Indeed, colonies of both species were similar or larger in mixed aggregates than in monospecific ones. The two species should preferentially meet and settle on a limited number of sites that are suitable for the aphids to feed on and grow.

If spatial displacement between species is an indicator of aphid competition (Dixon, 1992), the reverse statement is not necessarily true : the existence of still unoccupied shoots is not a sufficient criterion to rule out the possibility of interspecific competition, which could still occur at the occupied feeding sites. In this respect, the negative effect of the presence and/or development of one

species on the fitness of another one is a more relevant indicator of competitive interactions between coexisting species (e.g. MAY, 1973). At our study site, we failed to detect such effect as *B. brevipilosa* and *C. tuberculata* colony sizes were independent from each other in mixed aggregates. Additionally, in a given year, the presence on the same shoot of the numerically dominant species did not significantly alter the average colony size of the less abundant one.

This lack of correlation – and lack of evidence of direct competition – should however deserve further testing for a wider range of colony sizes of both species. All the combined observations of this study did not provide direct evidence of competition between *B. brevipilosa* and *C. tuberculata* though it is widely reported to occur among aphids (DENNO et al., 1995; DIXON, 1992). Studying additional sites and making surveys for more years would be needed to draw firm conclusions on the relationships between those two aphid species.

On the other hand, a number of factors unrelated to interspecific competition could account for the alternate outbreaks observed. The weight of predation in the shaping of aphid populations often remains unclear, but weather conditions and overall temperature are known to be major forces determining outbreak events in several well-studied species (DIXON, 1977, 1992). Differences in winter temperature in 2000 and 2001 may have favoured *C. tuberculata* in 2001 and *B. brevipilosa* in 2002. Indeed, the more precocious bud burst of the birch in 2002 (see Fig. 1) may have differentially influenced the population growth of the two species if they differ in their hatching dates, which could have dramatically influenced the outcome of their competitive interactions.

Additionally, the intrinsic properties of aphid life cycles could be a self-regulating mechanism causing the variation in abundance exhibited by aphid populations from year to year, in relation with fluctuations in the environmental conditions (DIXON, 1977, 1992). In short, the exhaustion of the host-plant and the crowding of aphids during high abundance seasons can lead to intense production of alates and massive emigration. The remaining aphids would be of smaller size, lower growth rate and fertility. Ultimately, this leads to a population crash for the rest of the season and for the following year due to the poor quality of autumn sexuals, until the aphids and the host plant recover (DIXON, 1975, 1977, 1992). A closer analysis of life history traits of *C. tuberculata* and *B. brevipilosa* with a concurrent survey of environmental factors, as well as experimental testing of the competitive interactions possibly occurring between the two species is now required to ascertain the driving forces leading to changes in abundance of local aphid populations.

ACKNOWLEDGEMENTS

We are greatly indebted to Anne-Michèle Warnier and Jean-Pierre Jansen from the Centre for Agronomic Research of Gembloux (Belgium) for aphid identification. S. Portha wishes to thank the Van Buuren foundation for financial support. This study was supported by the Belgian Fund for Joint Basic Research (grant n° 2.4510.01). C. Detrain is a research associate from the Belgian National Fund for Scientific Research.

REFERENCES

ATKINSON, M.D. (1992). *Betula pendula* Roth (*B. verrucosa* Ehrh.) and *B. pubescens* Ehrh. *J. Ecol.*, 80 : 837-870.

BERGESON, E. & F.J. MESSINA (1997). Resource- versus enemy-mediated interactions between cereal aphids (Homoptera : Aphididae) on a common host plant. *Ann. Entomol. Soc. Amer.*, 90 : 425-432.

DENNO, R.F., M.S. MCCLURE & J.R. OTT (1995). Interspecific interactions in phytophagous insects – Competition reexamined and resurrected. *Annu. Rev. Entomol.*, 40 : 297-331.

DIXON, A.F.G. (1975). Effect of population density and food quality on autumnal reproductive activity in the sycamore aphid, *Drepanosiphum platanoides* (Schr.). *J. Anim. Ecol.*, 44 : 297-304.

DIXON, A.F.G. (1977). Aphid Ecology : Life Cycles, Polymorphism, and Population Regulation. *Ann. Rev. Ecol. Syst.*, 8 : 329-353.

DIXON, A.F.G. (1992). *Aphid Ecology. An Optimization Approach.* Chapman & Hall, London.

GIANOLI, E. (2000). Competition in cereal aphids (Homoptera : Aphididae) on wheat plants. *Environ. Entomol.*, 29 : 213-219.

HAJEK, A.E. & D.L. DAHLSTEN (1986). Coexistence of three species of leaf-feeding aphids (Homoptera) on *Betula pendula*. *Oecologia*, 68 : 380-386.

LAWTON, J.H. & D.R. STRONG JR. (1981). Community patterns and competition in folivorous insects. *Am. Nat.*, 118 : 317-338.

MAY, R.M. (1973). *Stability and complexity in model ecosystems.* Princeton University Press, Princeton.

MESSINA, F.J., R. TAYLOR & M.E. KARREN (2002). Divergent responses of two cereal aphids to previous infestation of their host plant. *Entomol. Exp. Appl.*, 103 : 43-50.

PETERSEN, M.K. & J.P. SANDSTROM (2001). Outcome of indirect competition between two aphid species mediated by responses in their common host plant. *Funct. Ecol.*, 15 : 525-534.

Belg. J. Zool., 134 (1) : 89-96 March 2004

The distribution of ant nests (Hymenoptera, Formicidae) in coastal grey dunes of Flanders (Belgium) and their relationship to myrmecochorous plants

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ABSTRACT. During the summer of 2001, we conducted a study on the spatial distribution of ants in coastal grey dunes (Oostduinkerke, Western Flanders, Belgium). Nest locations of the most abundant ant species were analysed with multivariate techniques. *Tetramorium caespitum* frequented moss-dominated vegetation, whereas *Myrmica sabuleti, M. scabrinodis, Lasius flavus* and *L. meridionalis* preferred grassy vegetations. *Formica cunicularia* and *L. psammophilus* occurred in all types of grey dune vegetation. According to recent literature, a positive spatial relationship can exist between the positioning of ant nests and the location of the myrmecochorous plants *Viola curtisii*, *Luzula campestris* and *Polygala vulgaris* in coastal dunes. Neither preliminary investigations, nor our study could confirm this significant positive relationship : the occurrence of myrmecochorous plants seems to be independent of the proximity of nests. It is possible that relationships are masked by a high turnover rate of the nest sites or by a restricted seedling establishment. We did not, however, observe ants transporting seeds of *Viola*, *Luzula* or *Polygala* in the field, possibly indicating the inefficiency of searching for those seeds in areas where population densities of these plants are rather low and other food sources are abundantly available.

KEY WORDS : myrmecochory, *Polygala vulgaris, Luzula campestris, Viola curtisii.*

INTRODUCTION

Habitat characteristics are relatively well studied for most ant species in Western Europe (cf. Seifert, 1996), but few more detailed characterisations of nest location are available (Dekoninck & Bonte, 2002). However, the relationship with myrmecochores is unclear and not unambiguously documented. Myrmecochores are plants from which diaspores are transported by ants (Sernander, 1906). Most of them have a specialized structure, the elaiosome, containing a lot of fatty acids and providing up to one third of the total energy amount (Lisci et al., 1996). Several hypotheses have been put forward to explain the possible advantages of myrmecochory : escape from seed predation (Culver & Beattie, 1978; O'Dowd & Hay, 1980; Hanzawa et al., 1985; Ohara & Higashi, 1987), avoidance of competition with congeneric species (Handel, 1978; Higashi et al., 1989; Okhawara et al., 1996) and relocation of the seeds to favourable (nutrient-enriched) sites for germination and establishment (Culver & Beattie, 1978, 1980 and 1983; Davidson & Morton, 1981; Green et al., 1998). Rice & Westoby (1986) and Bond & Stock (1989), on the other hand, found that seed transport by ants in sclerophyll vegetation does not always result in deposition at nutrient enriched sites. Apparently, advantages for the plants of the transport of myrmecochore seeds by ants are not universal. It seems that the results are strongly dependent on the habitat type and the ant species involved.

Most studies of myrmecochory have been carried out in a variety of biotope types, such as temperate forests (Okhawara et al., 1996; Gorb & Gorb, 1998), mountain meadows (Culver & Beattie, 1983; Kovár et al., 2001), sclerophyll vegetation (Rice & Westoby, 1986; Bond & Stock, 1989) and deserts (O'Dowd & Hay, 1980). However, information about myrmecochory in coastal dunes is still scarce. According to Lack & Kay (1987), Oostermeijer (1989) and Kovár et al. (2001), a spatial relationship with these myrmecochores can be expected, since ants transport their seeds in laboratory experiments (Sernander, 1906). Kovár et al. (2001) found that myrmecochores had a non-random distribution in mountain grasslands, growing mainly on the edge of nests of *Lasius flavus*, *Tetramorium caespitum* and *Formica* spp. We wanted to compare the results of the study of Oostermeijer (1989) in the dunes of Terschelling (the Netherlands) with the situation in Oostduinkerke (Belgium). Oostermeijer (1989) demonstrated with mapping studies that the dispersal of seeds by ants has a marked effect on the distribution pattern of the standing population of *Polygala vulgaris* and *Viola curtisii*. Adult plants were found on or close to the active nest mounds of all ant species present, while growing sites of juvenile plants and seedlings were practically restricted to the nest environments. Dekoninck & Bonte (2002), however, did not detect a positive spatial relationship during a preliminary study in the Oostvoorduinen, part of our study site, for the same plant species and mainly the same ant species.

In this contribution, we characterised nest location in relation to the vegetation structure and the presence and abundance of the myrmecochores *Polygala vulgaris*, *Viola curtisii* and *Luzula campestris*. Our aim was not to

work out whether there is a causal relationship between the distribution of both ants and plants, but to describe possible spatial effects.

A perusal of the relevant literature reveals that all of the ant species in our study area show seed-carrying behaviour (SERNANDER, 1906; CULVER & BEATTIE, 1980 and 1983; OOSTERMEIJER, 1989).

MATERIAL AND METHODS

Study site

The research took place in the coastal dunes of Ter Yde (Oostduinkerke, West-Flanders, Belgium, Fig. 1), at three sites sharing a common geological history : 62.3 ha (with cattle grazing only in the non-studied part), 76.4 ha (with 16 sheep and 4 ponies) and 47.6 ha (3 ponies). Grey dunes and dense grasslands on lime-rich soils dominate the vegetation.

Coastal 'grey dune' is most readily defined using plant communities. Vegetation includes moss-dominated dunes as well as dune grassland (with a distinct organic soil layer) belonging to the *Cladonio-Koelerietalia* (PROVOOST et al., 2002). On the moss dunes, species such as *Tortula ruralis* or, in more fixed conditions, *Hypnum cupressiforme*, are dominant and accompanied by therophytes (*Crepis capillaris*, *Leontodon saxatilis*, …). In the grasslands, we find species such as *Asperula cynanchica*, *Potentilla erecta*, *Thymus pulegioides*, *Galium verum*, *Festuca rubra* and often a lot of *Avenula pubescens*.

Nest location as a function of vegetation structure and myrmecochores

During the summer of 2001, 59 plots (3 x 3 m^2 – quadrats) were placed around a randomly chosen ant nest. These plots were divided into nine quadrats of 1 x 1 m^2 (Fig. 2). This was done to check whether ants are restricted to a certain vegetation composition on both larger and smaller scales, and if so, whether there is a difference in their response towards both scales. For the relationship with myrmecochores, a look at these two scales seemed interesting to us because it is a simplified measure for the distance ant nest to plant; in each quadrat we can determine the presence and the amount of myrmecochores in relation to the presence or abundance of a certain ant species and the other way around. Since foraging range differs among ant species but usually does not exceed 1 m (GOMÉZ & ESPADALER 1998b), we chose 1 x 1 m^2 quadrats as our smallest space unit. We expect a spatial relationship to be found on a small scale (myrmecochores in direct vicinity of ant nests) but perhaps not on a larger scale (see OOSTERMEIJER (1989), who found all plants concentrated in a 20 cm-range of the nest). For each of the plots and quadrats, we assessed the coverage

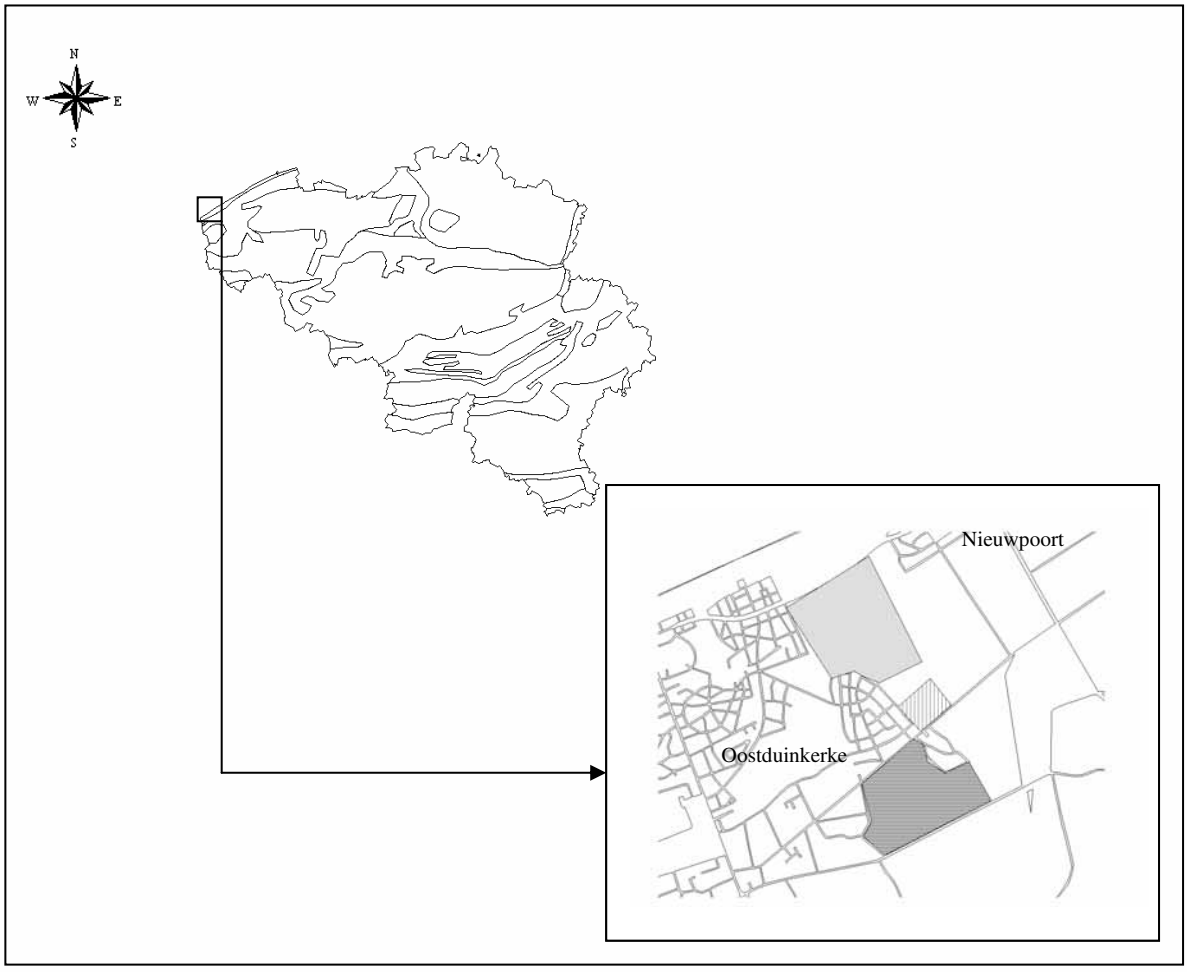

Fig. 1. – Study site. The shaded area indicates the zones studied.

of mosses, bare soil, shrubs and grasses and herbs (in%). For the herb-layer, three categories were used according to the vegetation-height (VLV=very low vegetation (1-3 cm), LV=low vegetation (3-20 cm), HV=high vegetation (> 20 cm)). We dug at about 20 (but up to 60, depending on population densities) random points per 3 x 3 m^2-quadrat. We sampled a 15 x 15 cm surface area to a depth of about 30 cm (most calices from *Lasius psammophilus* are at a depth of 10-30 cm, see SEIFERT 1996). Sampling places where more than ten workers occurred (see also SERRANO et al., 1993) or where males or larvae were found were mapped. One colony is spatially defined as the group of all contiguous spatial sampling places with presence of the same species (see also SERRANO et al., 1993). The percentage of undermined soil was then estimated on these maps using the surface area of the 'colonies'. This was necessary because it appeared impossible to discriminate between adjoining nests in *Tetramorium caespitum* and *L. psammophilus*, presumably because of calices leading to subterranean food resources (e.g. aphid-colonies).

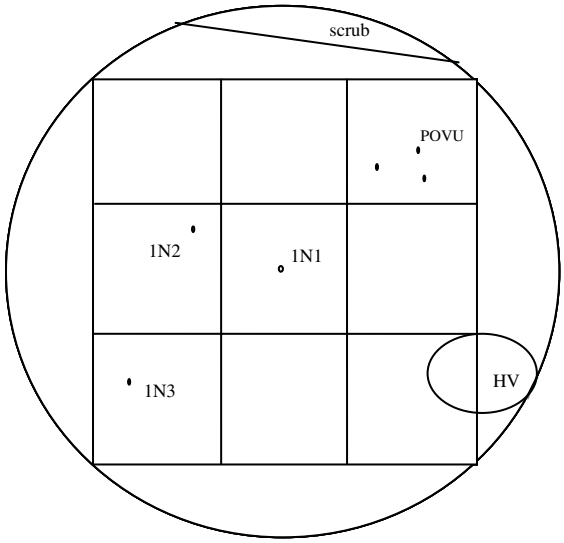

Fig. 2. – Study plot with indication of ant nests (1N1, 1N2 an 1N3), vegetation structures (Scrub; HV=high vegetation) an myrmecochores (here : POVU=*Polygala vulgaris*). A smal quadrat measures 1 x 1 m^2.

For each nest-unit, a sample of at least three workers was collected and identified using SEIFERT (1988a, 1988b, 1996 and 1997). The coverage of the myrmecochores *Luzula campestris*, *Polygala vulgaris* and *Viola curtisii* was also estimated and individual plants were indicated on the map (individuals could not be discerned for *L. campestris* because of its patchy distribution patterns caused by its clonal growth).

Data analysis

Multivariate analyses (DCA) were carried out with PC-ORD (version 4.20, MC CUNE & MEFFORD, 1997) to reveal overall trends in the ant data sets. The purpose here was to classify plots based on vegetation structure data, so that further analysis to examine the relationship between ants and myrmecochores could be performed, taking into account habitat preferences of both ant and plant species. Data on percentage of undermined soil per ant species and per plot were first put into perspective by dividing this percentage by the total percentage of undermined soil for each species (in all plots together). The data were Arcsin-transformed before performing the DCA, as is recommended for data expressed as a percentage (SOKAL & ROLPH, 1981). Pearson-correlation of the plot scores with vegetation structure data was used to explain the biological meaning of the axes. Pearson-correlations (SOKAL & ROLPH, 1981) were also used to test co-correlation between vegetation structure and the abundance of myrmecochores.

Univariate tests were performed using the statistical program STATISTICA (version 6.0, STATSOFT, 1994) with a significance level taken at 0.05. A Mann-Whitney-U-test was used to check the existence of a possible relationship between the abundance of certain ant species and the presence of the myrmecochorous plant species. Chi^2-tests (SIEGEL, 1956) were performed to analyse absence/presence trends between myrmecochores and ant species. Bonferroni corrections were used in case of multiple comparisons.

RESULTS

General results

Twelve species were found in the study area, with *Lasius psammophilus* the most abundant species, followed by *Tetramorium caespitum*, *Formica cunicularia* and *Myrmica sabuleti* (Table 1). The social parasite *Lasius meridionalis* was also well represented (27.8% of the nests of *Lasius psammophilus*, the host species, were 'infected' by *L. meridionalis*).

TABLE 1

List of species and their frequency in the study area.

Species	# plots of 59 (%)
Formica cunicularia Latreille 1798	18 (30.5)
Lasius psammophilus Seifert 1992	36 (61.0)
Lasius niger (Linnaeus 1758)	7 (11.9)
Lasius fuliginosus (Latreille 1798)	1 (1.7)
Lasius meridionalis (Bondroit 1919)	10 (16.9)
Lasius flavus (Fabricius 1781)	7 (11.9)
Myrmica sabuleti Meinert 1860	16 (27.1)
Myrmica scabrinodis Nylander 1846	5 (8.5)
Myrmica specioides Bondroit 1918	4 (6.8)
Myrmica rugulosa Nylander 1846	1 (1.7)
Myrmica rubra (Linnaeus 1758)	1 (1.7)
Tetramorium caespitum (Linnaeus 1758)	26 (44.1)

Nest location in relation to vegetation structure

DCA-ordination revealed three relevant axes, explaining variation in nest-location of ants as a function of the vegetation structure. The percentage of variance explained by these factors was rather low (17.4%, 16.2% and 10.5% for axis 1, 2 and 3 respectively), indicating a quite high variance in the samples. Still, trends can be observed in the results of the DCA (Fig. 3).

However, no linear relations with the first axis were found (Table 2). Along this axis, one 3 x 3 m^2-quadrat, in which *L. niger* was abundantly present, was separated from

the rest. *L. psammophilus* was the only accompanying species in this plot, not exceeding an undermined soil surface of 1%. The ant species composition in this plot was thus very different from the others. The second axis was significantly positively correlated with the moss coverage and negatively with the herb layer and the abundance of *Polygala vulgaris* (but there was a strong positive correlation between the abundance of POVU and LV, see further). The third axis was only significantly negatively correlated with the estimated ground coverage of *Polygala vulgaris* (Table 2). Correlations with vegetation cover characteristics along this axis were not significant after Bonferroni-correction, but indicate biologically relevant relations.

Tetramorium caespitum was found in plots with a lot of mosses, in contrast to *Myrmica sabuleti* and *M. scabrinodis*, which were found in grassy vegetation. *Lasius flavus* and *L. meridionalis* also seemed to prefer grasslands, but they differed from the *Myrmica*-species, which were found in plots with higher vegetation and without *Polygala vulgaris*. *M. specioides* seems to prefer a habitat with a lot of mosses, but was sometimes found in grasslands as well. Nests of *L. niger* were often found in disturbed patches, both with mosses and herbs/grasses. *Formica cunicularia* and *L. psammophilus* showed no clear preference for any grey dune vegetation structure (Fig. 3).

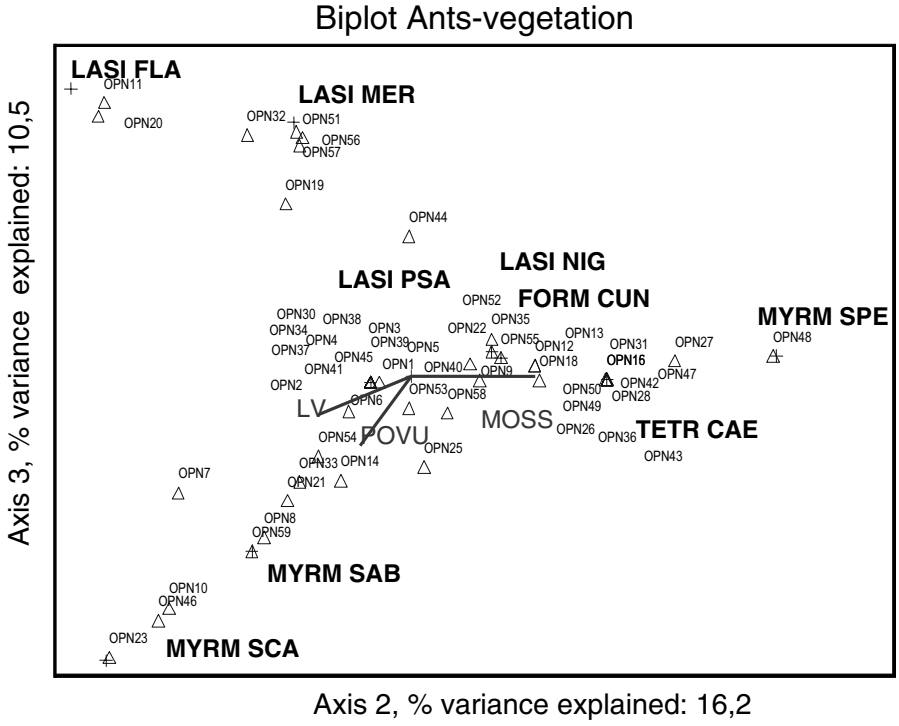

Fig. 3. – DCA-biplot ant nest location as a function of the vegetation structure. Species that were found in only one plot were omitted. Triangles indicate the plot number ('opn' 1-59), + -symbols indicate ant species. Components : λ_1=0.99, λ_2=0.92, λ_3=0.60. Total inertia : 5.70.
Legend : MOS=mosses; LV=low vegetation (mainly herbs, up to 20 cm); POVU=*Polygala vulgaris*; MYRM SCA=*Myrmica scabrinodis*; MYRM SAB=*Myrmica sabuleti*; MYRM SPE=*Myrmica specioides*; TETR CAE=*Tetramorium caespitum*; FORM CUN=*Formica cunicularia*; LASI NIG=*Lasius niger*; LASI PSA=*Lasius psammophilus*; LASI MER=*Lasius meriodionalis*; LASI FLA=*Lasius flavus*.

TABLE 2

Pearson-correlation-coefficients between the ordination scores (Fig. 3) and the estimated vegetation coverage and coverage of the myrmecochores *Viola curtisii* (VICU), *Luzula campestris* (LUCA) and *Polygala vulgaris* (POVU). Abbreviations : HV=high vegetation (> 20 cm); LV=low vegetation (3-20 cm); VLV= very low vegetation (< 3 cm); MOSS=mosses; SAND=bare soil; SHRU=shrub; total cover=HV + LV + VLV + SCRU + MOSS; herb layer=HV + LV + VLV. Bold : p < 0.0045 (significant after Bonferroni-correction). * : p < 0.05 (NS after Bonferroni-correction).

	HV	LV	MOSS	SHRU	VLV	SAND	Total cover	Herb layer	VICU	LUCA	POVU
Axis 1	0.2053	-0.0059	-0.151	0.268*	-0.045	-0.058	0.058	0.142	-0.038	0.146	-0.053
Axis 2	-0.2835*	**-0.515**	**0.592**	0.014	0.260	0.100	-0.100	-0.580	.174	-0.227	**-0.388**
Axis 3	0.3145*	-0.332*	0.0093	-0.0234	-0.003	0.076	-0.077	-0.028	0.1315	-0.060	**-0.443**

T. caespitum and *L. psammophilus*, the two most abundant species, were rarely found together in the same plot (χ^2=11.21, p < 0.001), except in grass-plots (in three of the four cases where *T. caespitum* was present, *L. psammophilus* was also found (χ^2, p > 0.05)).

Nest location in relation to the distribution of myrmecochores

The finding that the presence or the abundance of *Polygala vulgaris* could be an important factor determining ant nest location (or the other way around : ants determine plant distribution) has to be interpreted with caution since the myrmecochores studied each have their own habitat preferences as well. In Figure 3, one can see that *M. sabuleti* and *M. scabrinodis* are indicative for low-vegetation grasslands, often in the presence of *Polygala vulgaris*, whereas *L. flavus* and *L. meridionalis* are found in grasslands with a higher vegetation and without this plant species.

Polygala vulgaris was only present in short grazed grasslands and there was a strong positive correlation between the cover-percentage of this myrmecochore and the percentage of low vegetation (R=+ 0.36, p < 0.001). The same is true for *Viola curtisii* and the coverage of bare soil (R=+ 0.38, p < 0.001) and for *Luzula campestris* and the low and higher herb layer (respectively R= + 0.64 and R=+ 0.45; both p < 0.001). In the case of *Polygala vulgaris* and *Luzula campestris*, there is also a significant negative correlation with the moss-coverage (respectively R=-0.45; R=-0.63; both p < 0.01).

On the largest scale (3 x 3 m^2), a significant positive association was found between the presence of *L. psammophilus* (χ^2-test, p=0.025) and *M. sabuleti* respectively (χ^2-test, p=0.047) with the presence of *L. campestris* in moss dunes but this association was lacking in grassland vegetation. This result again suggests that vegetation structure is more important than the presence of myrmecochores per se. At a scale of 2 x 2 m^2 and 1 x 1 m^2, the relationships were no longer significant, except for all plots together (both grassland and moss dunes). *Myrmica scabrinodis* showed a marginally significant positive association in its presence with the presence of *Polygala vulgaris* (χ^2-test, p=0.047). For the other ant and plant species, there was no significant relationship at any spatial scale in terms of presence/absence at different scales.

Besides a lack of significant association in terms of presence/absence between ant species and myrmecochores, the abundance of several ant species per plot (measured by the percentage of nest cover) was also not significantly influenced by the presence of myrmecochores (Mann-Whitney-U-tests, p > 0.05).

DISCUSSION

Nest location in relation to vegetation structure

According to the DCA ordination, species have distinctive habitat preferences, even within grey dunes. The vegetation structure (coverage, height) is an important factor determining the habitat of several species, as was earlier mentioned by Boomsma & Van Loon (1982), Handelmann (1997); Blomquist et al. (2000); Lopéz et al. (2000) and Dekoninck & Bonte (2002). Our results on habitat preferences are in agreement with the literature (Assing, 1986; Van Boven & Mabelis, 1986; Seifert, 1996; Boer & De Gruyter, 1999; Boer, 2001 and Dekoninck & Bonte, 2002).

We believe that *T. caespitum* is an even earlier pioneer than *L. psammophilus*, as both were rarely found together in the same plot, which could be an indication of habitat segregation because of interspecific competition, with *T. caespitum* being the first pioneer established in dry moss dune vegetation, followed by the colonisation of the more fixed habitat by *L. psammophilus*. This confirms the findings of Boomsma et al. (1987) who noted the presence of *T. caespitum* on all 17 of the Frisian Islands, while *L. psammophilus* was only found on Texel, the biggest island, near the mainland, indicating *T. caespitum* is a more mobile species or a better survivor.

Nest location in relation to the distribution of myrmecochores

Our analyses do not indicate the existence of a relationship between ant nest location of any ant species and the presence of myrmecochores. The association between the myrmecochore *P. vulgaris* and *M. scabrinodis* is probably the result of co-variation with vegetation structure and can neither be rejected nor confirmed.

Oostermeijer (1989) documents a significant relationship between the location of the myrmecochores *V. curtisii, L. campestris* and *P. vulgaris* on the one hand, and the position of ant nests of *L. niger*, *T. caespitum*, *F. fusca*, *F. rufibarbis* and *M. schencki* on the other hand. In Terschelling, this author found most of the myrmecochorous plants on or in the close proximity (20 cm) of the nests, independently of the ant species involved. Our results do not confirm this. Earlier, Dekoninck & Bonte (2002) also did not find a positional ant-plant relationship in the Oostvoorduinen, as was the case in our study. They worked with the same species as the ones found in our study area. The spatial relationship between ants and myrmecochores can be two-sided :

Firstly, the spatial distribution of ant nests could be influenced by the presence and the amount of myrmecochores (the availability of the nutritious seeds). In this case, one would expect ant nests to be more abundant in the proximity of myrmecochores, which was not the case in our study. Vegetation structure seemed much more important in determining the presence of certain ant species than the presence of myrmecochores. We suppose that ants search for their food along trial ways, and take all the food they meet on their path (see also Mull & MacMahon, 1997). Since population density of the myrmecochores studied was rather low in our study site, we think ants take seeds that are abundantly available (e.g. *Phleum arenarium* grass-seeds) instead of searching for seeds of these myrmecochores. We suggest that the availability of the seeds is an important factor because ants took myrmecochorous seeds under laboratory conditions (Lehouck et al., unpubl.), which shows that seed carrying by all ant species in our study area is possible, as mentioned in literature (e.g. Sernander, 1906). Culver & Beattie (1978) state that the chance to encounter ant-dispersed plant species could make a 'behavioural' differ-

ence, in that dispersion is much less efficient in areas where ant-dispersed plant species are rather scarce. Although Lack & Kay (1987) supposed the existence of a possible search image, this has never been confirmed, but availability may play an important role here as well. In our study area there was enough food to provide ant colonies with the energy needed : insects, non-myrmecochorous seeds, honeydew, nectar, … and most ants are not strictly granivorous, nectivorous or insectivorous. They have a combined diet (Alonso, 2000). It is possible that the presence of myrmecochores determines ant nest distribution in areas with a dense population of myrmecochores (as was the case in the study of Oostermeijer (1989), who performed his study in a moist dune valley) or low food availability. According to Kjellsson (1985), dispersion-efficiency of myrmecochorous seeds clearly depends on the acute requirement for food in an ant colony, as well as the distance of the food source to the nest.

Secondly, the location of the myrmecochorous plants could be determined by seed transport by ants. As a consequence, one should expect plants to grow in the close proximity of ant nests. In our study, this was not the case. Data were analysed on several spatial scales and significant trends at the largest scale (3 x 3 m^2) were often not significant at smaller scales (2 x 2 m^2, 1 x 1 m^2). Several authors mention the concentration of myrmecochorous plants on ant nests (Sernander, 1906; Davidson & Morton, 1981; Oostermeijer, 1989), while others find them on the edge of the nests (Kjellsson, 1985; Oostermeijer, 1989; Goméz & Espadaler, 1998a; Gorb & Gorb, 1998; Kovár et al., 2001). This suggests that elaiosomes are removed, while seeds can germinate in 'refuse piles'. Even if the whole seed is initially taken into the nest, it is possible that the seed with the discarded elaiosome is removed afterwards (Lack & Kay, 1987). Culver & Beattie (1983) even found a negative relationship between the position of myrmecochorous plants and ant nests of *Formica canadensis* in a mountain meadow in Colorado, USA (no plants on ant nests). They thought that establishment of seedlings could be restricted chemically (see also Seifert 1996) or mechanically. Pathogenic infection is also thought to be increased (Christensen, 1972 in : Kjellsson, 1985). Since Oostermeijer (1989) did find seedlings on nests in coastal dunes, one could say that these arguments are probably not valid in our case (i.e. with these particular plant and ant species, both in Terschelling and Oostduinkerke). However, there are some 'small', but important, differences between the two study areas. In our study area, there have been no rabbits for the last ten years, because of a serious epidemic of myxomatosis. Seed predation by rabbits is therefore not applicable in our study site. Seed burial by ants protects the seeds against predation (e.g. O'Dowd & Hay 1980). Since rabbit grazing is intensive in Terschelling (pre-dispersal predation of 70% of the seeds of *Viola* and *Luzula*), it is possible that seeds germinate in ant nests but not in the surroundings in the study area of Oostermeijer (1989), whereas germination is possible everywhere in our study site. The absence of rabbits in our study would mean that germination of myrmecochores is not restricted by predation by rabbits, nor in the nests, nor in the surroundings. This could explain the absence of any positive relationship in our study. Next, a behavioural difference in seed transport by ants of the same species in different studies could be more apparent than real. Habitat is likely to play an important role in the outcome (Culver & Beattie, 1978). The slight habitat differences between the study of Oostermeijer (1989) and our study (moist dune valley versus grey dunes) could make differences in ant behaviour and germination chances through differences in soil conditions and infection rate by pathogens. The first can explain why ants do not choose to make their nests in close proximity to myrmecochorous plants, whereas the latter could be an explanation for the fact that myrmecochores were not found on/in the vicinity of ant nests in our study. It is strange, however, that Oostermeijer (1989) found nest translocation to be infrequent (no translocation observed within five years). According to Culver & Beattie (1978), the fact that ants frequently abandon their nests may be crucial for the germination of the seeds. They found that seed scarification was an opportunity for germination but if seeds, especially during germination, are subject to a continuing disturbance by ants – excavating more tunnels or gnawing new shoots – this advantage is likely to be lost. Certain authors mention a high turn over rate of nests. Culver & Beattie (1978) found that 15 out of 23 nests of *Aphaenogaster* sp. moved within 11 days; *L. niger* is known to shift nest sites regularly (Smallwood & Culver, 1979). This nest site translocation could cause the absence of a spatial relationship, especially when considering adult plants instead of seedlings : movement of the nests is then faster than the time between the collection of the seeds and seedling emergence. Moreover, all three myrmecochores studied are perennials (*P. vulgaris* can reach an age of 5-10 years (Lack & Kay, 1987)). Dekoninck & Bonte (2002) thought nest site translocation could explain the absence of a spatial relationship in the Oostvoorduinen. In the area they studied, turnover rate of nest sites is high, probably because of disturbance by intensive cattle grazing (Dekonink & Bonte, unpubl.). However, the cause of nest site translocation is still not understood (competition, parasitism, disturbance, … – cf. Gordon, 1992 and Smallwood & Culver, 1979) and data on residence time of nests are often confusing : what exactly is a nest? *L. niger* for instance, has multiple nest sites with movements among them (see Smallwood & Culver (1979) and Culver & Beattie (1980) for a brief discussion). The nest site turnover is different in different ant species and in different studies, suggesting both a species specific and a condition-related effect (presence of parasites, environmental conditions, etc.). There is no overall pattern. Mapping experiments using permanent quadrats and behavioural experiments (cafeteria experiments in the field and in the laboratory) could be useful in further research to help in understanding these complex interactions between ants, myrmecochores and external/internal factors influencing the turnover rate of nest sites.

As a conclusion, we can say that our results about spatial relationships between ant nests and myrmecochores are not in accordance with the results of Oostermeijer (1989) in Terschelling. Whereas Oostermeijer (1989) found a significant positive spatial relationship, this was not the case in the coastal dunes of Ter Yde (Oostduinkerke, Belgium) : ant nests were not concentrated in plots where the myrmecochorous plants were abundant, nor

was there any special relationship within the plots. Slightly different habitat characteristics (in terms of infection rate by parasites, soil conditions, nest site turnover and availability of other food resources) could explain these different outcomes, taking into account both the chance for seedlings to establish on ant nests and the frequency of nest movements by ants.

REFERENCES

Alonso, L.E. (2000). Ants as indicators of diversity. In : *Ants : Standard methods for measuring and monitoring biodiversity.* Agosti, D., J. Majer, E. Alonso & T. Schultz (Eds.). Biological Diversity Handbook Series, Smithsonian Institution Press, Washington D.C. and London : 80-88.

Assing, V. (1986). Distribution, densities and activity patterns of the ants (Hymenoptera : Formicidae) of *Calluna* heathlands in northwestern Germany. *Entomol. Gen.,* 11 (3/4) : 183-190.

Blomquist, M.M., H. Olff, M.B. Blaauw, T. Bongers & W.H. van der Putten (2000). Interactions between above- and belowground biota : importance for small-scale vegetation mosaics in a grassland ecosystem. *Oikos,* 90 : 582-598.

Boer, P. (2001). Duinmieren en mierenduinen. *Duin,* 24 (4) : 15-16.

Boer, P. & T. de Gruyter (1999). *Mieren in de Noord-Hollandse duinen. Verspreidingsatlas. BOO-onderzoeksrapport.* Provinciaal Waterleidingbedrijf Noord-Holland, Bloemendaal (23 pp).

Bond, W.J. & W.D. Stock (1989). The costs of leaving home – Ants disperse myrmecochorous seeds to low nutrient sites. *Oecologia,* 81 : 412-417.

Boomsma, J.J. & A.J. van Loon (1982). Structure and diversity of ant communities in successive coastal dune valleys. *J. Anim. Ecol.,* 51 : 957-974.

Boomsma, J.J., A.A. Mabelis, M.G.M. Verbeek & E.C. Los (1987). Insular biogeography and distribution ecology of ants on the Frisian islands. *J. Biogeogr.,* 14 (1) : 21-37.

Culver, D.C. & A.J. Beattie (1978). Myrmecochory in *Viola* : dynamics of seed-ant interactions in some West Virginia species. *J. Ecol.,* 66 : 53-72.

Culver, D.C. & A.J. Beattie (1980). The fate of *Viola* seeds dispersed by ants. *Am. J. Bot.,* 67 : 710-714.

Culver, D.C. & A.J. Beattie (1983). Effects of ant mounds on soil chemistry and vegetation patterns in a Colorado montane meadow. *Ecology,* 64 : 485-492.

Davidson, D.W. & S.R. Morton (1981). Competition for dispersal in ant-dispersed plants. *Science,* 213 : 1259-1261.

Dekoninck, W. & D. Bonte (2002). Beïnvloeden mierennesten de verspreiding van planten in matig voedselrijke duinen? Resultaten uit Oostduinkerke. *Natuur.focus,* 1 (2) : 61-64.

Goméz, C. & X. Espadaler (1998a). Seed dispersal curve of a Mediterranean myrmecochore : influence of ant size and the distance to nests. *Ecol. Res.,* 13 : 347-354.

Gomez, C. & X. Espadaler (1998b). Myrmecochorous dispersal distances : a world survey. *J. Biogeogr.,* 25 : 573-580.

Gorb, S.N. & E.V. Gorb (1998). Semennoj material moeravejnikov kak resoeltat potoka semjan na territorijach *Formica polyctena* Fabr. vsjirokolistvennom lesoe=Seed pool of anthills as the result of seed flow on *Formica polyctena* Fabr. territories in a broadleaved forest. *Russian J. Ecol.,* 29 (1) : 55-62.

Gordon, D.M. (1992). Nest relocation in harvester ants. *Ann. Entomol. Soc. Am.*, 85(1) : 44-47.

Green, W.P., D.E. Pettry & R.E. Switzer (1998). Formicarious pedons, the initial effect of mound-building ants on soils. *Soil Survey Horizons,* 39 (2) : 33-44.

Handel, S.N. (1978). The competitive relationship of three woodland sedges and its bearing on the evolution of ant dispersal of *Carex pedunculata*. *Evolution*, 323 : 151-163.

Handelmann, D. (1997). *Dune insects-grasshoppers (Saltatoria) and ants (Formicidae). Coastal dunes. Management, protection and research.* Report from a European seminar, Skagen, Denmark (4 pp).

Hanzawa, F.M., A.J. Beattie & A. Holmes (1985). Dual function of the elaiosome of *Corydalis aurea* (Fumariaceae) – Attraction of dispersal agents and repulsion of *Peromyscus maniculatus,* a seed predator. *Am. J. Bot.,* 72 (11) : 1707-1711.

Higashi, S., S. Tsuyazaki, M. Ohara & F. Ito (1989). Adaptive advantages of ant-dispersed seeds in the myrmecochorous plant *Trillium tschonoskii* (Liliaceae). *Oikos,* 54 : 389-394.

Kjellsson, G. (1985). Seed fate in a population of *Carex pilulifera* L. 1. Seed dispersal and ant-seed mutualism. *Oecologia,* 67 : 416-423.

Kovár, P., M. Kovárová, P. Dostal & T. Herben (2001). Vegetation of ant-hills in a mountain grassland : effects of mound history and of dominant ant species. *Plant Ecol.,* 156 : 215-227.

Lack, A.J. & O. Kay (1987). Genetic structure, gene flow and reproductive ecology in sand-dune populations of *Polygala vulgaris*. *J. Ecol.,* 75 : 259-276.

Lisci, M., M. Bianchini & E. Pacini (1996). Structure and function of the elaiosome in some angiosperm species. *Flora,* 191 (2) : 131-141.

López, F., F.J. Acosta & J.M. Serrano (2000). Asymmetric interactions between plants and seed-harvesting ants in a Mediterranean pasture. *Ecol. Res.,* 15 : 449-452.

McCune, B. & M.J. Mefford (1997). *Multivariate analysis of ecological data.* Gleneden Beach, Oregon : MjM Software.

Mull, J.F. & J.A. MacMahon (1997). Spatial variation in rates of seed removal by harvester ants (*Pogonomyrmex occidentalis*) in a shrub-steppe ecosystem. *Am. Midl. Nat.,* 138 : 1-13.

O'Dowd, D.J. & M.E. Hay (1980). Mutualism between harvester ants and a desert ephemeral : escape from rodents. *Ecology,* 61 : 531-540.

Ohara, M. & S. Higashi (1987). Interference by ground beetles with the dispersal by ants of seeds of *Trillium* species (Liliaceae). *J. Ecol.,* 75 : 1091-198.

Ohkawara, K., S. Higashi & M. Ohara (1996). Effects of ants, ground beetles and the seed-fall patterns on myrmecochory of *Erythronium japonicum* Decne (Liliaceae). *Oecologia,* 106 : 500-506.

Oostermeijer, J.G.B. (1989). Myrmecochory in *Polygala vulgaris* L., *Luzula campestris* (L.) DC. and *Viola curtisii* Forster in a Dutch dune area. *Oecologia,* 78 : 302-311.

Provoost, S., C. Ampe, D. Bonte, E. Cosyns & M. Hoffmann (2002). Ecology, management and monitoring of dune grasslands in Flanders, Belgium. In : *Littoral 2002. The Changing Coast.* EUROCOAST (Ed.). Eurocoast/EUCC, Porto : 11-22.

Rice, B. & M. Westoby (1986). Evidence against the hypothesis that ant-dispersed seeds reach nutrient-enriched microsites. *Ecology,* 67 : 1270-1274.

Seifert, B. (1988a). A taxonomic revision of the *Myrmica* species of Europe, Asia minor, and Caucasia (Hymenoptera, Formicidae). *Abh. Ber. Naturkundemu.*, 62(3) : 1-75.

Seifert, B. (1988b). A Revision of the European Species of the Ant Genus *Chthonolasius* (Insecta, Hymenoptera, Formicidae). *Ent. Abh. Mus. Tierk. Dr.,* 51(8) :143-180.

Seifert, B. (1996). *Ameisen, beobachten, bestimmen.* Naturbuch verlag, Augsburg and Berlin (352pp).

Seifert, B. (1997). *Formica lusatica* n. sp. – a sympatric sibling species of *Formica cunicularia* and *Formica rufibarbis*

(Hymenoptera, Formicidae). *Abh. Ber. Naturkundemu.,* 69(5) : 3-16.

Sernander, R. (1906). *Entwurf einer Monographie der Europäischen Myrmekochoren.* Kungliga Svenska Vetenskapsakademiens Handlinger. Band 41.N17, 41/7 (410 pp).

Serrano, J.M., F.J. Acosta & F. Lopez. (1993). Belowground space occupation and partitioning in an ant community during succession. *Eur. J. Entomol.,* 90 : 149-158.

Siegel, S. (1956). *Nonparametric statistics for the behavioral sciences*. McGraw-Hill Book Company Inc., New York-Toronto-London (312 pp).

Smallwood, J. & D.C. Culver (1979). Colony movements of some North American ants. *J. Anim. Ecol.*, 48 : 373-382.

Sokal, R.R. & F.J. Rohlf (1981). *Biometry : the principles and practice of statistics in biological research*. 2nd edition. Freeman, San Francisco (859 pp).

Statsoft (1994). Statistica for Windows. Tulsa : OK. Statsoft, Inc.

van Boven, J.K.A. & A.A. Mabelis (1986). *De mierenfauna van de Benelux (Hymenoptera : Formicidae)*. Wetenschappelijke mededelingen van de KNNV 173 (64 pp).

Belg. J. Zool., 134 (1) : 97-103 March 2004

Do season and habitat influence the behaviour of Haflinger mares in a coastal dune area?

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ABSTRACT. This study was performed to gain more knowledge about the behaviour and habitat use of Haflinger mares, free-ranging in a low-productivity dune area. Detailed data on these animals' time budgets were collected over a full year, through the focal animal observation technique. On average the Haflinger horses spent 68% of the daytime grazing, 18% resting and 8% walking. Seasonal features influenced horses' behaviour, mainly through a change in grazing time. Shorter grazing time in summer allowed the animals to rest longer than during the other seasons. We suggest that especially the decreased forage quality and quantity of the grazed habitats in the non-growing season account for the increased grazing time in autumn and winter. In all four seasons the horses preferred grazing in the grassy habitat. However, habitat use showed seasonal variation. Grey dunes were grazed more intensively in winter and spring, compared to summer and autumn. The contribution of roughage, scrub and woodland to the habitat use was low over the entire year. For several response variables the observed variation could be partly explained by the differences between individual animals.

KEY WORDS : horse, free-ranging, habitat use, time-budget, grazing behaviour, non-grazing behaviour.

INTRODUCTION

Several authors have reported (daylight or 24 hours) time-budgets of feral horses (Salter & Hudson, 1979; Jarrige & Martin-Rosset, 1987) or free-ranging horses living in natural or semi-natural conditions (Duncan, 1980; Duncan, 1985; van Dierendonck et al., 1996; Berger et al., 1999; Boyd & Bandi, 2002). On the whole, time-budgets of free-ranging and feral horses show large similarities, with highest time-investment in grazing. Resting, moving and alertness take most of the remaining time. However, behavioural differences due to environmental conditions, such as habitat, forage quality and weather are reported, as well as a relationship with intrinsic aspects such as age, sex and reproductive state.

The aim of the present study was to describe the behaviour and the habitat use of Haflinger horses, introduced into an old coastal dune area with low primary production. This low-productivity environment offers the herbivores rather low levels of forage quality and quantity, in comparison with more nutrient-rich systems. These nutrient and energy restrictions are even more pronounced during the non-growing season, i.e. the season with low plant production (from October to March in temperate regions). Free-ranging herbivores have to make many foraging decisions at different resolution levels (Senft et al., 1987; Stuth, 1991), resulting in a foraging strategy that meets the large herbivores' nutrient and energy requirements. These decisions are primarily made in relation to forage availability and quality, which are in turn determined by environmental conditions. We expect that the rather low levels of forage quality and quantity will be reflected in the foraging behaviour of the Haflinger horses, in particular by long grazing times. Furthermore, we suppose that the horses adapt their behaviour and habitat use to the seasonal changes in their environment. According to the literature we may assume that this adaptation will result in an increased grazing time as well as a broader habitat use outside the growing season.

MATERIAL AND METHODS

Study site and animals

Research was performed in the nature reserve Ghyvelde (60 ha), an old dune area close to the northern French coastline and bordering an equally old dune ridge in Belgium (Adinkerke). Ghyvelde is located in a coastal region with mild winters and mild summers. Mean annual temperature is 9.8˚C. The average minimum temperature of the coldest month (January) is –7.2˚C, average maximum temperature of the hottest month (August) is 28.4˚C. Mean annual precipitation is min 520 mm and max 870 mm. In summer, autumn, winter and spring mean monthly precipitation is 60.7 mm, 74.8 mm, 56.5 mm and 48.5 mm, respectively (means over the period 1963-2002) (Meteo WVL vzw).

Two thirds of the area is covered by open habitat, mainly formed by *Carex arenaria*-dominated grassland (*Plantagini-Festucion* community), alternating with grey dunes, dominated by mosses and lichens and a sparse cover of grasses and forbs (*Thera-Airion* community). One central afforested area and several dispersed, small patches of trees shape the woodland at the site (approximately 23% of the area). Approximately 7% of the area is scrub vegetation, consisting of *Hippophae rhamnoides, Ligustrum vulgare, Salix repens* and *Sambucus nigra*.

During the study, a herd of 14 to 18 Haflinger horses grazed the site. They were introduced to decrease or hamper the encroachment of competitive plant species that tend to form species poor to monospecific vegetations. They graze year round and no additional food is given. The horses have access to one artificial water point for drinking. We chose three adult mares as the focal animals for the observations : one had a foal, the other two were non-lactating. All three mares were in good condition.

Behavioural observations

Data were collected through continuous focal animal observation (ALTMANN, 1974). From May 2000 until April 2001 we conducted 31 sessions of six hours. All observations took place during daylight (between 9:00h and 19:00h) and were done by one observer. During a six-hour period we continuously monitored the behaviour of one focal animal, chosen at random from the three mares that were a priori selected for this study. Most of the horses are habituated to man and can be approached within a range of 1 m without causing any visually observable influence on behaviour.

We recorded the duration (accuracy : 1 s) of the different behavioural types, as well as vegetation type and vegetation height. We registered and took into account grazing as well as non-grazing behaviour (drinking, walking, standing alert, resting upright, laying down, rolling, grooming, mutual grooming, defecating, urinating). To analyse the data the different vegetation types considered in the field were lumped into five habitat types : 'grassy vegetation', 'grey dune', 'roughage', 'scrub' and 'woodland', which cover 35%, 32%, 3%, 7% and 23% of the area respectively. For vegetation height we used a scale related to the animal's physiognomy : 'no height' (in case of no vegetation), 'shortly grazed', 'hoof', 'knee', 'belly', 'spine' and 'higher'. We have no data on the relative availability of each of these height classes. Season definition follows the plant productivity periods in temperate regions, i.e. summer (June-August), autumn (September-November), winter (December-February) and spring (March-May).

Data analysis

The calculation of the time-budget was based on the total time spent per day on each behaviour. The correlation between the total time of the different behaviours was analysed separately for each individual mare (since we found individual variation). A Bonferroni correction (adjustment of the p-value with k=3) was performed to draw conclusions about the correlations for the three mares. Pearsons correlations were calculated if data were distributed normally; if not, we used Spearman correlations.

Variation in the time-budget was investigated by the use of the following response variables : mean time per day spent in a certain behaviour, mean number of bouts, mean number of periods of a certain behaviour per day, mean duration of a bout and mean duration of a period of a certain behaviour. A 'bout' is a phase in which a certain behaviour is performed without interruption. A 'period' is the accumulation of several bouts of the same behaviour if they are not interrupted for more than five minutes. For example, the horse can stop a grazing bout to scan its environment. After a few seconds or minutes it can prolong its grazing behaviour and stop this to start a resting period. That grazing period (called a 'meal') consists of two bouts. The short interruption is not seen as a break of the meal, but is not included in the calculation of the meal duration, which is only the effective grazing time during a meal. Main attention focussed on the behavioural types grazing, resting and walking. Additionally, we considered standing alert, grooming, mutual grooming, drinking, defecating, urinating and rolling. We investigated whether the observed variation in the response variables was affected by seasonality. We were aware of the possibility that differences in behaviour between individual animals could explain, at least partly, the observed variation. Therefore, we used mixed-model ANOVA to investigate the effect of the fixed factor 'season' on the variation in mean time, mean number of bouts and mean bout duration, and included the random factor 'individual' into the model. If the random factor was not significant, we consequently excluded it from the model. The Scheffé multiple comparison procedure was used as post hoc test. In case of inconsistency with the assumptions for the use of ANOVA, we used Kruskal-Wallis One Way Analysis. However in such cases we could not incorporate a random factor. This meant that for the analysis of the effect of the factor 'season', the impact of possible individual differences could not be regarded. Secondly, we had to analyse the potential effect of 'individual' with a separate analysis.

To investigate the habitat use of the horses we considered the variable mean grazing time per day per habitat type or per vegetation height. When on a given day an animal was not grazing in a certain habitat or height, null values were included to calculate the mean grazing time. In the ANOVA-model we considered two fixed factors 'season' and 'habitat type' or 'height category', their interactions and the random factor 'individual'. We eliminated a non-significant random factor or interaction from the final model. We investigated the use of the five different habitat types a second time by taking into account the availability of the five habitat types. Therefore we multiplied the mean grazing time per day per habitat type with a correction factor, derived from the relative occupancy of the habitat types.

All analyses were performed using SPSS 11.0 for Windows.

RESULTS

Time-budget

Table 1 gives an overview of the time budget of the three Haflinger mares. In general grazing took the main part of the time-budget; on average 68% of the observed time. On average, the horses spent 18% of their daytime resting, 8% walking and 3% standing alert. Grooming, drinking, nursing, mutual grooming, defecating, urinating, rolling and interactions accounted for only 4% of the total daytime. Fig. 1 illustrates the time-budget over the whole year and the variation between seasons. For each of the three mares we found a significant negative correlation between total grazing time and total resting time per

TABLE 1

Time (minutes) per 6 hours day and number of bouts per 6 hours of each behaviour: mean, minimum (min.), maximum (max.) and Standard Error (SE). Sample size: 3 individuals; 31 observation days.
Fixed effect of season and random effect of individual (ind.) on mean time per day and mean number of bouts were investigated: ***: p<0.005; **: p<0.01; *: p<0.05; n.s.: not significant

	Time				Effect		Number				Effect	
Behaviour	**Mean**	**Min.**	**Max.**	**SE**	**season**	**ind.**	**Mean**	**Min.**	**Max.**	**SE**	**season**	**ind.**
Grazing	242.6	128.3	291.0	16.1	*	***	92	44	112	5.9	n.s.	n.s.
Resting	63.8	25.5	153.2	12.0	***	***	9	3	29	2.4	n.s.	***
Resting up	59.4	7.3	152.3	12.8			9	2	28	2.5		
Lying down	4.4	0.0	25.8	2.3			1	0	3	0.3		
Walking	27.9	15.5	37.4	2.1	n.s.	n.s.	76	56	91	3.4	n.s.	n.s.
Standing alert	12.3	3.3	33.2	2.9	n.s.	**	25	8	45	4.2	n.s.	n.s.
Grooming	4.0	0.9	9.1	0.8	n.s.	n.s.	11	5	17	1.4	***	***
Drinking	2.0	4.2	4.8	0.4	n.s.	n.s.	2	1	5	0.4	n.s.	n.s.
Mutual grooming	1.4	0.0	6.3	0.6	n.s.	n.s.	2	0	7	0.6	n.s.	n.s.
Defecating	0.8	0.2	1.9	0.1	n.s.	n.s.	4	1	6	0.5	n.s.	*
Urinating	0.6	0.2	1.0	0.1	n.s.	n.s.	3	1	5	0.4	n.s.	n.s.
Rolling	0.1	0.0	0.4	0.0	n.s.	*	0.4	0	1	0.1	n.s.	n.s.
remainder	0.1											

day. We could not conclude this for the three mares together, since the significant correlation did not remain after Bonferroni correction (p=0.057).

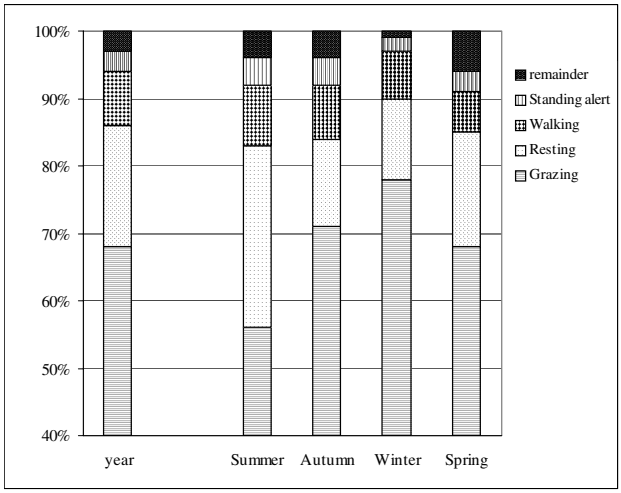

Fig. 1. – Time-budget of the Haflinger horses over the entire year, and in summer, autumn, winter and spring. Percentages are based on mean time spent per day.

Grazing behaviour and habitat use

Mean grazing time per day was affected by season (p=0.030). The random factor individual could not be deleted from the statistical model as it had a significant effect. Post-hoc tests showed that summer had a significantly lower mean grazing time compared to autumn and winter (Su : 56% of six hours; Au : 71%; Wi : 78%; Sp : 68%).

Average duration of a meal, average duration of a grazing bout, average number of meals and average number of grazing bouts were not different in the four seasons. However, the observed variation in meal duration, grazing bout duration, number of meals and number of grazing bouts could be explained to a certain extent by the differences between individual animals.

To investigate the habitat use of the horses we considered the differences in average grazing time per habitat type per day. The horses grazed 176 min/6 hrs in grassy vegetation and 54 min/6 hrs in grey dunes. In comparison, grazing times in other habitat types were much lower : 2, 7 and 4 minutes in roughage, scrub and woodland, respectively. Table 2a illustrates the ANOVA results : significant main effect of habitat ($p < 0.001$), significant interaction season x habitat ($p < 0.001$) and a significant random effect. Similar results were found when we analysed the habitat use with the correction for habitat availability (grazing time in grassy habitat : 167 min/6 hrs; grey dune : 60 min; roughage : 19 min; scrub : 33 min; woodland : 6 min)(Table 2b; Fig.2). The significant interaction illustrates the seasonal changes in habitat use. Grey dunes were grazed more in winter and spring than in summer and autumn, and this was at the expense of the grassy habitat. Roughage was only foraged in autumn. In autumn, winter and spring scrub was grazed a bit more, compared to summer. The woodland was visited for grazing a bit more often, compared to the other seasons. Nonetheless, the contribution of roughage, scrub and woodland to the habitat use was low, throughout the year.

TABLE 2

Results of the mixed-models ANOVA examining the effects of the fixed factor 'Habitat type', 'Season', the interaction, and the random factor 'Individual' on the variable Grazing Time.
a) without correction for availability of the habitat types. B) with correction for availability of the habitat types

		df_1	df_2	F	P
a	Habitat	4	33	152.634	**<0.001**
	Season	3	33	0.880	0.462
	Habitat*Season	12	33	4.347	**<0.001**
	Individual (Random)				significant
b	Habitat	4	33	69.149	**<0.001**
	Season	3	33	1.739	0.179
	Habitat*Season	12	33	2.988	**0.006**
	Individual (Random)				significant

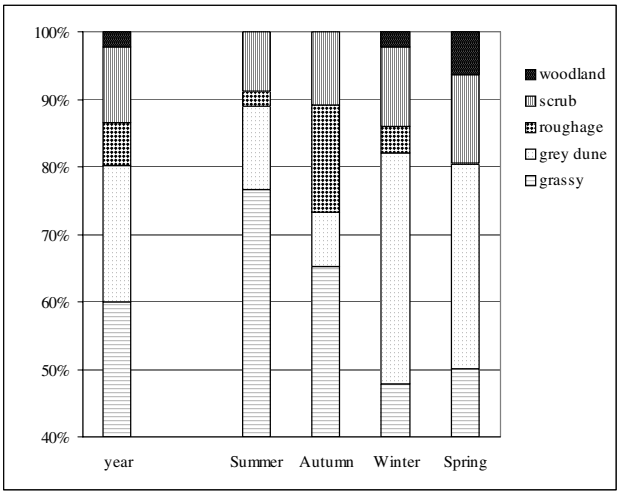

Fig. 2. – Habitat use of the Haflinger horses over the entire year and in summer, autumn, winter and spring. Percentages are based on mean time grazing per day, corrected for the availability of the five distinguished habitat types.

We analysed the effect of vegetation height on grazing time when the horses were foraging in grassy habitat and grey dune. The Haflinger mares were grazing in hoof high vegetation 57% of the time that they were grazing in grassy habitat or grey dune, and 40% in shortly grazed vegetation. This difference seemed more pronounced in summer and spring than in autumn and winter, so we also analysed if there was a significant interaction between the effect of height and the effect of season. There was a significant effect only of height ($p=0.029$). No significant interaction or significant random effect of individual was found.

Resting behaviour

The mean resting time per day was significantly different between seasons ($p=0.005$) and between individual animals. In summer significantly more time was spent resting compared to autumn, winter and spring (result of post hoc-tests) (Su : 27% of six hours; Au : 13%; Wi : 12%; Sp : 17%). The duration of a resting period and the duration of a resting bout were similar in all seasons and for all individuals. The factor season had also no effect on the average number of resting periods. Number of resting periods and number of resting bouts were significantly different between individual animals. Resting behaviour was only observed in grassy vegetations and grey dunes, never in roughage, scrub or woodland.

Walking behaviour

The mean walking time per day was not affected by the factor season. In summer, autumn, winter and spring the Haflinger mares on average walked respectively 33 min, 30 min, 24 min and 22 min/6 hours. Individual horses did not differ in mean walking time per day. There were no seasonal or individual differences in the average duration of a walking period, average number of walking periods, average walking bout and average number of walking bouts. Horses mostly walked in the grassy vegetations and grey dunes, and rarely moved around in roughage, scrub or woodland.

Other behavioural aspects

We considered here the behaviours standing alert, grooming, mutual grooming, drinking, urinating, defecating and rolling. We found no seasonal variation in the mean time per day spent on these behaviours. For the behaviours standing alert and rolling we found significant individual differences. The mean grooming frequency per day was significantly different between seasons ($p=0.004$) and between individuals. Individual variation was also found for the mean defecating frequency. The mean time of a bout was different between seasons for defecating and different between individual horses for grooming.

DISCUSSION

Time-budget

On average, the Haflinger horses spent 68% of the daytime grazing and 18% resting, of which only 1% was lying down. The horses were walking around for 8% of their time and spent 3% standing alert. This daylight time-budget is in line with time-budgets of other free-ranging and feral horses. Jarrige & Martin-Rosset (1987) reported that feral horses spend 50-73% of their time grazing during daylight. Przewalski horses in a nature reserve in the Mongolian steppes only grazed an average of 49% of the daytime (van Dierendonck et al., 1996). Duncan (1985) concluded that feeding of Camargue horses generally occupies 50-70% of a whole day and resting 20-30%, the remainder being spent in alertness and movement. We suggest that the rather long grazing times of the Haflinger horses reflect the poor nutritive quality and quantity of the grazed habitats. Berger (1986) reported high grazing times (68.3% & 78.1% for non-reproductive and reproductive mares) in low quality home ranges as opposed to lower grazing times (58.5% & 65.8% for non-reproductive and reproductive mares) in high quality home ranges.

We found low daily resting times, and resting occurred mainly in the standing position. As Duncan (1985), Mayes & Duncan (1986) and Pratt et al. (1986) already indicated for other horse breeds, we consider it very probable that the Haflinger horses also rest more at night, in the standing as well as in a recumbent position, than during the day. Paradoxical sleep occurs in the recumbent resting periods (Boyd, 1998; Waring, 2003); however, standing, not recumbency, is the posture of minimal energy demand for horses (Winchester, 1943). Environmental factors influence the horse's resting behaviour (Waring, 2003), while individual variation has been reported as well (Duncan, 1980). However, we believe that there is a minimum level for resting critical to equid well-being, as also suggested by Duncan (1992). Increased resting time above this threshold is possible when other maintenance requirements are fulfilled. In nutrient-poor systems horses will be more time-limited, in comparison with horses in nutrient-rich systems, owing to the increased foraging effort needed to meet their energy and nutrient requirements. We suggest that on the one hand the maximum grazing time of horses is determined by a threshold for other maintenance activities, in particu-

lar resting. On the other hand "free" time to increase the resting time is mainly determined by the time spent on the horses' main activity, i.e. grazing. Since the Haflinger horses forage in a nutrient-poor system, we hypothesise that even if the horses rest more at night, the proportion of the time spent resting in a 24-hour period would remain low, in comparison with other studies (Duncan, 1985; Boyd, 1998). Furthermore, diet is one of the factors affecting patterns of sleep. Stabled horses increased their total time lying down when fed on a higher quality diet (Dallaire & Ruckebush, 1974). Duncan (1985) found a positive correlation between time spent lying and protein concentration in the diet. The Haflinger horses were mainly foraging on grassland dominated by *Carex arenaria*, which has indeed a low protein content, especially in the non-growing season (Cosyns, unpubl.).

Seasonal variation in time-budget

During autumn and winter the horses increased their grazing time, while in summer feeding time dropped to a minimum. This is in line with previous studies in temperate regions (Duncan, 1985; van Dierendonck et al., 1996; Berger et al., 1999; Cosyns et al., 2001; Menard et al., 2002), as well as in subarctic conditions (Salter & Hudson, 1979). We suggest that especially the relatively higher quality and availability of forage in summer accounted for the drop of grazing time compared to the non-growing seasons. Horses perform most of their foraging behaviour during the daylight period (Duncan, 1985; Pratt et al., 1986). Therefore, we might expect that in autumn and winter the grazers had to concentrate their grazing more in a shorter daylight period, than in summer and spring, when they can spread their grazing activities over a longer daylight period. Although this could partly explain the increased daylight grazing time in autumn and winter, we also find this pattern in studies which have calculated time-budgets based on observations spread over twenty-four hour periods (Duncan, 1985; Berger et al., 1999; Menard et al. 2002). Thermoregulation during hot summer days could result in more grazing during the late evening or night. However, we rarely observed horses seeking shade. Therefore we assume that this factor was of minor importance in explaining the seasonal variation in daylight grazing time. Some authors have suggested that the observed drop in foraging time in summer is mainly caused by a response to attacks by biting flies (Duncan, 1985; Mayes & Duncan, 1986), which is also seen in reindeer (Hagemoen & Reimers, 2002). Though we did not measure this parameter, we think that biting insects are not present at the study site in such numbers that they would influence the horses' behaviour strongly. The lack of seasonal variation in grazing bout duration and number of grazing bouts could reflect the lack of disturbance by external factors, such as biting flies. Concluding, as mentioned above, we suggest that seasonal differences in forage quality and quantity play a major role in the seasonal variation in grazing time of the Haflinger mares in the present study. Grazing time is generally lowest when forage is abundant and of good quality, and highest when forage is of low quality or availability is limited (Vallentine, 1990; Stuth,1991). Duncan (1985) suggested that horses increased their feeding time in winter to a maximum possible value in an attempt to maintain a high quality diet. Lamoot *et al*. (unpubl.) found longer grazing times, but lower bite rates, in autumn and winter compared to summer and spring, for donkeys and ponies. At the level of the grazed patch, a prolonged searching time for plants or plant parts to be consumed to achieve a diet of acceptable quality, might increase the grazing time (and diminish the bite rate).

The Haflinger horses in the present study spent more time resting per day in summer, in comparison with the other seasons, mainly as a result of the (non-significantly) higher number of resting periods in summer. There was no seasonal variation in walking time per day. As discussed above, we assume that the increased resting time in summer was related to the decreased grazing time in summer. In summer the grazing horse could meet its nutritional requirements more easily and in less time. Consequently, this resulted in "free" time available to spend resting. Seasonal variation in resting time and the lack of seasonal variation in walking time are not in line with the findings of Duncan (1985). He found longer walking times in summer, and little seasonal variation in time spent resting. This might be due to the differences between study sites. In our study site palatable patches are available in a more or less continuous pattern. Therefore, seasonal variation in walking time is not expected. In the Camargue insect harassment in summer could result in more moving around. We suggest that insects are not present in our study site in such numbers that they would influence the horses' behaviour strongly.

Seasonal variation in grooming frequency per day was found, with more grooming bouts in spring, which could be related to the moulting season, as was also suggested by Tyler (1972). We did not find differences between seasons for any of the other behaviours considered. Mean frequency of drinking at Ghyvelde was 2.1 time per 6 hours. Feral horses are reported to drink only once or twice in a 24 h period (Fraser, 1992). At pasture, frequency, but not duration of drinking bouts increased as temperature increased (Crowell-Davis *et al*., 1985), a phenomenon not found in the present study. Kimura (1998) reported seasonal variation in mutual grooming, probably due to changes in distances between individual horses. No seasonal differences in mutual grooming behaviour were found in the present study. Although we did not measure distances between horses, our field observations did not indicate remarkable seasonal changes in individual spacing.

Habitat use

Taking in account the availability of the distinguished habitat types, we found that the horses grazed, over the entire year, mostly in grassy habitat, i.e. grasslands dominated by *Carex arenaria.* However, the habitat use of the Haflinger horses showed seasonal variation. In winter and spring grey dunes were grazed more intensively than in summer and autumn. The grassy habitat was grazed less intensively in winter and spring. The contribution of roughage, scrub and woodland to the habitat use was poor over the entire year, although there was a limited use of scrub that remained constant over the entire year. A slightly increased use of roughage was observed in autumn, and woodland was used a little more in spring.

When grazing grassy habitat and grey dune, the mares grazed significantly more in patches with 'hoof' height, compared to shortly grazed patches. This figure did not provide any indications on preferences, however, as there are no data about the relative availability of the different vegetation heights. We hypothesised that the Haflinger horses would show seasonal variation in habitat use, which is confirmed by our results. However, we expected that the horses would graze more in scrub and woodland during the non-growing season, due to the depletion of the preferred grassy habitat. It remains unclear why the Haflinger horses did graze more in grey dunes, and not in woodland or in scrub. A possible reason could be the presence of a relatively large number of winter annuals in these grey dunes, which might serve as relatively good quality winter forage. Nonetheless, the total primary production of these winter annuals remains very low. Our results are in line to some extent with the findings of Gordon (1989), who investigated vegetation community selection on the Isle of Rhum (Scotland). Out of four different ungulates (cattle, red deer, goat and pony) ponies performed the smallest seasonal changes in vegetation use. Only in autumn ponies broadened their vegetation community use. Pratt et al. (1986) reported that grasslands remained of major importance throughout the year for New Forest ponies, which is consistent with our results, but the ponies showed a greater flexibility in foraging behaviour over the winter months. Especially woodland was grazed more in winter. Also Duncan (1985) concluded that the Camargue horses were more dispersed over the various vegetation complexes in the cooler season.

Variation among individual horses

In the Camargue the time-budgets of free-ranging horses were investigated over several years (Duncan, 1980). On the basis of the differences in time-budget, he could divide the animals into three groups, e.g. adult females, yearlings and adult males. However, the overall picture was one of remarkably similar investments of time in all activities, especially with regard to foraging time.

Prior to the present study period we selected three adult mares for observation. Consistent with the findings of Duncan (1980) and because the horses were foraging as a herd, we did not expect far-reaching differences in time-budget between the mares. However, for the analysis we wanted to take into account possible variation among individuals, especially because we noticed during observations that one mare, older and presumably high on the dominance rank, was grazing less than the other two. Our results demonstrate that the time-budgets indeed differed between the observed mares. We suggest that bias through individual variation could be avoided to some extent by increasing the number of focal animals for the data collecting through the focal animal observation technique. The individual variation in time-budgets has far-reaching consequences for data analysis. When investigating environmental differences in behavioural aspects, one has to keep in mind that variation between observed individuals can bias the results, if not incorporated in the statistical analyses. In the present study we aimed to investigate seasonal variation in time budgets. Using ANOVA-models we could take into account the role of the random factor 'individual' on the observed variation. In some cases the outcome of the test changed, if we tried the analysis without this random factor, which illustrated its importance. This opportunity is not available in non-parametric tests. Generally, in cases where assumptions for parametric test are not met, a non-parametric alternative is used. It is now questionable which choice is the best : violating assumptions or not taking into account variation due to a random factor? Again, we suggest the need for a large sample size when investigating the behaviour of a herd of horses.

CONCLUSIONS

The Haflinger mares performed time-budgets similar to the ones presented in literature, with grazing as the main time-investment. They showed rather long grazing times, which could be a response to their low-productivity habitat. Seasonal features influenced horses' behaviour, mainly through a change in time spent grazing. The drop in grazing time in summer made time available for resting. Most of their grazing, as well as their non-grazing behaviour, took place in *Carex arenaria*-dominated grassland, with short sward height, and this during the entire year. In winter and spring grey dunes were grazed to a greater extent, compared to summer and autumn. Although not expected, individual variation explained at least partly the observed variability of many variables.

ACKNOWLEDGEMENTS

We would like to thank Le Conservatoire du Littoral, Le Direction du Service des Espaces Naturels and Conseil Général du Nord for permission to conduct research in the nature reserve Ghyvelde. We thank S. Vanacker and G. Van Spaendonk for the support of the Access database, and Prof. L. Lens for his remarks on the used statistics. I. Lamoot has a grant supplied by FWO Flanders (Foundation of scientific research- Flanders).

REFERENCES

Altmann, J. (1974). Observational study of behaviour : sampling methods. *Behaviour*, 49 : 227-267.

Berger, A., K.M. Scheibe, K. Eichhorn, A. Scheibe & J. Streich (1999). Diurnal and ultradian rhythms of behaviour in a mare group of Przewalski horse (*Equus ferus przewalskii*), measured through one year under semi-reserve conditions. *Appl. Anim. Behav. Sci*., 64 : 1-17.

Berger, J. (1986). *Wild horses at the Great Basin. Social Competition and Population Size.* Wildlife Behavior and Ecology Series, The University of Chicago Press (326 pp).

Boyd, L. & N. Bandi (2002). Reintroduction of takhi, *Equus ferus przewalskii*, to Hustai National Park, Mongolia : time budget and synchrony of activity pre- and post-release. *Appl. Anim. Behav. Sci*., 78 : 87-102.

Boyd, L.E. (1998). The 24-h time budget of a takh harem stallion (*Equus ferus przewalskii*) pre- and post-reintroduction. *Appl. Anim. Behav. Sci*., 60 : 291-299.

Cosyns, E., T. Degezelle, E. Demeulenaere & M. Hoffmann (2001). Feeding ecology of Konik horses and donkeys in Belgian coastal dunes and its implications for nature management. *Belg. J. Zool*., 131 : 111-118.

Crowell-Davis, S.L., K.A. Houpt & J. Carnevale (1985). Feeding and drinking behaviour of mares and foals with free access to pasture and water. *J. Anim. Sci*., 60 : 883-889.

Dallaire, A. & Y. Ruckebusch (1974). Sleep and wakefulness in the housed pony under different dietary conditions. *Can. J. Comp. Med.,* 38 : 65-71.

Duncan, P. (1980). Time-budgets of Camargue horses. II. Time budgets of adult horses and weaned sub-adults. *Behaviour*, 72 : 26-49.

Duncan, P. (1985). Time-budgets of Camargue horses. III. Environmental influences. *Behaviour*, 92 : 188-208.

Duncan, P. (1992). *Horses and grasses : The nutritional ecology of equids and their impact on the Camargue.* Ecological Studies 87. Springer Verlag, New York (287 pp).

Fraser, A.F. (1992). *The Behaviour of the Horse*. CAB International, Wallingford, UK.

Gordon, I.J. (1989). Vegetation Community Selection by ungulates on the Isle of Rhum. II. Vegetation community selection. *J. Appl. Ecol*., 26 : 53-64.

Hagemoen, R.I.M. & E. Reimers (2002). Reindeer summer activity pattern in relation to weather and insect harassment. *J. Anim. Ecol*., 71 : 883-892.

Jarrige, R. & W. Martin-Rosset (1987). *Le Cheval : reproduction, selection alimentation, exploitation*. INRA, Paris.

Kimura, R. (1998). Mutual grooming and preferred associate relationships in a band of free-ranging horses. *Appl. Anim. Behav. Sci*., 59 : 265-276.

Mayes, E. & P. Duncan (1986). Temporal patterns of feeding behaviour in free-ranging horses. *Behaviour*, 96 : 105-129.

Menard, C., P. Duncan, G. Fleurance, J.-Y. Georges & M. Lila (2002). Comparative foraging and nutrition of horses and cattle in European wetlands. *J. Appl. Ecol.*, 39 : 120-133.

Pratt, R.M., R.J. Putman, J.R. Ekins & P.J. Edwards (1986). Use of habitat by free-ranging cattle and ponies in the new forest, Southern England. *J. Appl. Ecol*., 23 : 539-557.

Salter, R.E. & R.J. Hudson (1979). Feeding Ecology of Feral Horses in Western Alberta. *J. Range Manage*., 32 : 221-225.

Senft, R.L., M.B. Coughenour, D.W. Bailey, L.R. Rittenhouse, O.E. Sala & D.M. Swift (1987). Large herbivore foraging and ecological hierarchies. *Bioscience,* 37 : 789-799.

Stuth, J.W. (1991). Foraging Behaviour. In : *Grazing management : an ecological approach*. Heitschmidt X. & Stuth X. (Eds.). Timber Press, Inc., Portland, Oregan : 65-83.

Tyler, S.J. (1972). The behaviour and social organisation of the New Forest ponies. *Anim. Behav. Monogr.*, 85-196.

Vallentine, J.F. (1990). *Grazing management*. Academic Press, San Diego (533 pp).

van Dierendonck, M.C., N. Bandi, D. Batdorj, S. Dügerlham & B. Munkhtsog (1996). Behavioural observations of reintroduced Takhi or Przewalski horses (*Equus ferus Przewalskii*) in Mongolia. *Appl. Anim. Behav. Sci*., 95-114.

Waring, G.H. (2003). *Horse Behavior*. Noyes publications/William Andrew Publishing, New York (442 pp).

Winchester, C.F. (1943). Energy cost of standing in horses. *Science,* 97 : 24.

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Belg. J. Zool., 134 (1) : 105-108 March 2004

Catfish with distaste for preservatives

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ABSTRACT. The effect of food quality on the electrodetection performance of two catfish, *Ictalurus nebulosus* (LeSueur, 1819), was assessed in a two-alternative forced-choice experiment while the fish were performing at threshold level. Three different types of food were administered, namely minced beef paste (N), minced beef paste with 0.7% Butylparaben (B), and fish food pellets (P). When fed and rewarded with minced beef paste the fish made a maximum number of correct choices. Minced beef paste with 0.7% Butylparaben reduced the response performance to approximately 50 %. When fish food pellets were used, the performance returned to maximum again. It is concluded that such conditioning experiments are suitable for quantifying the aversiveness of food.

KEY WORDS : *Ictalurus nebulosus,* electroreception, preservative, automated feeding, welfare.

INTRODUCTION

This experiment deals with the effect of automated feeding dispensers on the response behaviour of electrosensitive catfish, *Ictalurus nebulosus,* in a two-alternative forced-choice experiment. In earlier experiments on the detection threshold of the electrosensory system in catfish, the reward was delivered as protein-rich paste, distributed via a peristaltic pump through a silicon tube with a diameter of 3 mm (Peters et al., 1995a; Peters et al., 1995b; Peters et al., 1996; Peters et al., 1999; Eeuwes et al., 2001). The advantage of this system is that food can be delivered under water in any quantity needed. However, the drawback is that the food spoils rather fast and has to be refreshed every one or two days. In order to improve the manner of food distribution, we tested the effect of adding a preservative to the protein-rich paste, and the administration of dry fish food pellets.

MATERIAL AND METHODS

Animals

Two specimens of freshwater catfish (*Ictalurus nebulosus)*, one female (115 gram and 180 mm, referred to as fish BB) and one male (73 gram and 160 mm, referred to as fish BC), were the subjects of this experiment. The catfish were obtained from Visplant (Numansdorp, The Netherlands). They were kept in glass tanks filled with copper-free tap water at Utrecht University until the experiments commenced. During the experiments fish BB was kept in a glass aquarium (91 x 28 cm, water height 9 cm) and fish BC was housed in a similar glass aquarium (91 x 28 cm, water height 8.5 cm). These aquaria were connected to a buffer tank, from which water was circulated and filtered. The total water volume was 180 litres. Once a week, the water was partially refreshed. The tanks were placed in an air-conditioned room, and the temperature of the water was kept at 17± 2˚C with a cooling device. Initially the tanks were filled with copper-free tap water, conductivity 0.25-0.33 mS. The conductivity increased by approximately 0.01 mS in the course of the experiments due to excretions of the fish and feeding. A 12h dark-12h light regime ensured that experiments were only performed during the most active period of the fish, i.e. at night.

Protocol

The electrodetection threshold was determined for each fish in a number of threshold sessions. The catfish were subjected to one session a night. A single session consisted of 100 trials. 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 as the fish, attached to the wall of the tank (Fig. 1). If the fish stayed underneath its shelter for two seconds, the light was switched off and a weak uniform direct current field was presented.

In the uniform fields, the side at which the anode was located was alternated semi-randomly. If the fish interrupted the infrared bundle nearest to the cathode, food was distributed by the food dispenser at that site, followed by 30 s of dark feeding time. 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.

At all times, the light above the test tank operated as a negative reinforcer. After a correct choice (choosing the cathode), the strength of the following stimulus was decreased by 1 dB. After a false choice the following stimulus was increased by 3 dB. A trial was marked a no-go trial when fish did not make a choice within ten minutes. In this case, the stimulus did not change in strength. The steps up and down were not equal (respectively 3 dB and 1 dB) because if so, the stimulus would remain 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., 1995b).

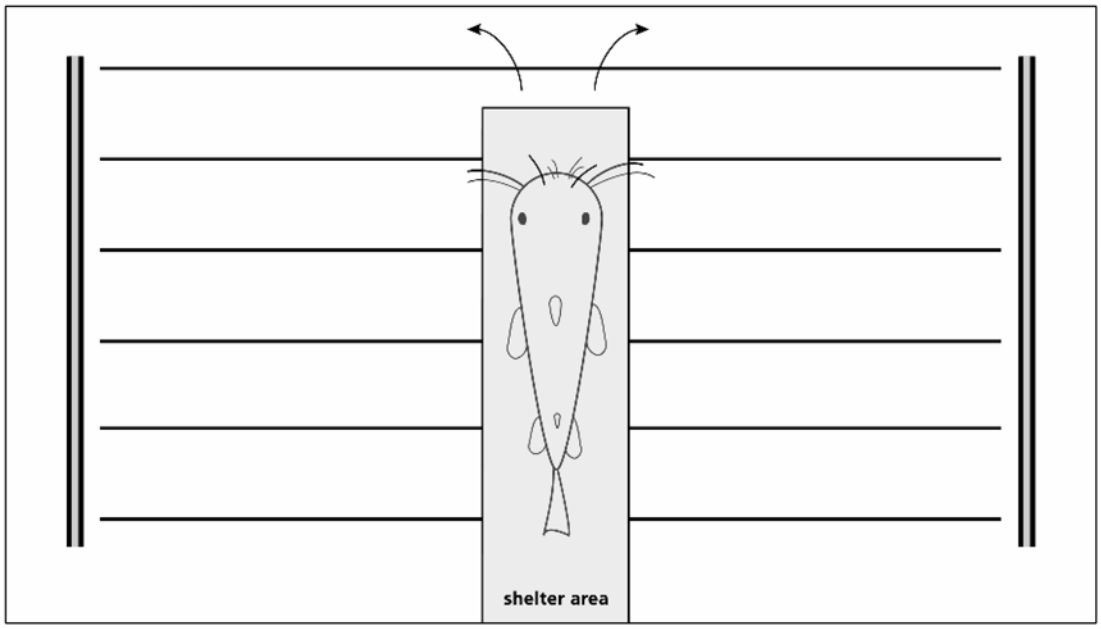

Fig. 1. – Schematic drawing of the experimental tank, top view. Vertical bars represent the strip electrodes. Horizontal lines represent the field lines during a trial. The shelter area provides protection for top lights (not shown) and serves as a dwelling space between trials. Several centimetres from the electrodes infrared detectors are placed on the outside of the tank. At the same position a plastic bar is placed at the bottom of the tank to provide a tactile stimulus for the catfish. This position is marked as the decision point. Food dispensers are placed between decision point and electrodes.

Stimulation

Stimuli were generated by a LAB-PC data acquisition card (National Instruments). The stimulus was fed into a homemade 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.

Shaping

Before the actual testing started, the fish was subjected to a period of shaping. In this period the stimulus protocol differed. The anode location was switched from one side to the other after each trial. The field strength (60-350µV/cm) was certainly within the perceptive range. As soon as the fish performed at a 90% level, the anode location in uniform fields was randomised with a maximum of three in succession at the same side. When the level of correct choices was 90% or over, the experiment was initiated (Peters & Van Wijland, 1974).

Data analysis

Threshold has been defined as the stimulus strength that could be maintained 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 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 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 preceded by more than three correct choices. If a single session yielded more than one threshold value, only the lowest of these values was used in further analysis.

Statistical analysis was performed per individual, since the variance between the two specimens was large. Hence, each catfish was used as its own control. Differences in threshold values, correct choices, false choices, and no-go trials between minced beef paste with (B) and without Butylparaben (N) were analysed using the Mann-Whitney U test.

Composition of food

Three types of food were tested : (i) Food paste composed of 60 g beef, 1.5 g Trouvit elite response fish food, 1.5 g agar-bacto/gelatin (1:1), and 125 ml water. This minced beef paste has been used on a regular basis in conditioning experiments. (ii) The same concoction with 0.7% Butylparaben (from 10% in 95% alcohol). Butylparaben is an antimicrobial and antifungal preservative commonly used in cosmetics, foods, and pharmaceuticals. The used concentration was based on a preliminary study (Klaver, unpubl.). (iii) Trouvit fish food pellets, eel 2 mm (Trouw, Putten-NL). The minced beef paste, both with (B) and without Butylparaben (N), was delivered via peristaltic pumps and plastic tubing, and a hydraulically-driven syringe delivered the fish food pellets (P). The pellet distribution mechanism showed some imperfections and therefore not all correct choices of the catfish were rewarded with a fish food pellet. However, in such an instance, the succeeding correct choice was properly rewarded.

RESULTS

The responses of fish BB and BC to changing food types and distribution system are presented in Fig. 2. In both fish, the effects of these changes are seen best in the percentage of correct choices and no-go trials.

In fish BB, every deviation from the 'normal' food type, i.e. that used during shaping, resulted in an increase of no-go trials and decrease of correct choices ($p << 0.001$, N compared to B). Performance was immediately restored when the fish was rewarded with food (N) again. The electrodetection threshold slowly rose ($p = 0.002$, N compared to B) when Butylparaben was added to the food, whereas the number of correct choices immediately decreased. Apparently, the number of no-go trials increased at the cost of correct choices. This pattern was repeated at the second administration of Butylparaben (B). As the food type and distribution were changed more radically; the fish pellet dispenser was installed, the electrodetection threshold instantly rose by a factor of twenty-five. Initially, the percentage of no-go trials barely increased, and the percentage of correct choices dropped dramatically. However, after six sessions**,** it performed at its usual threshold level again. This indicates that the fish experienced difficulties in understanding the new set-up.

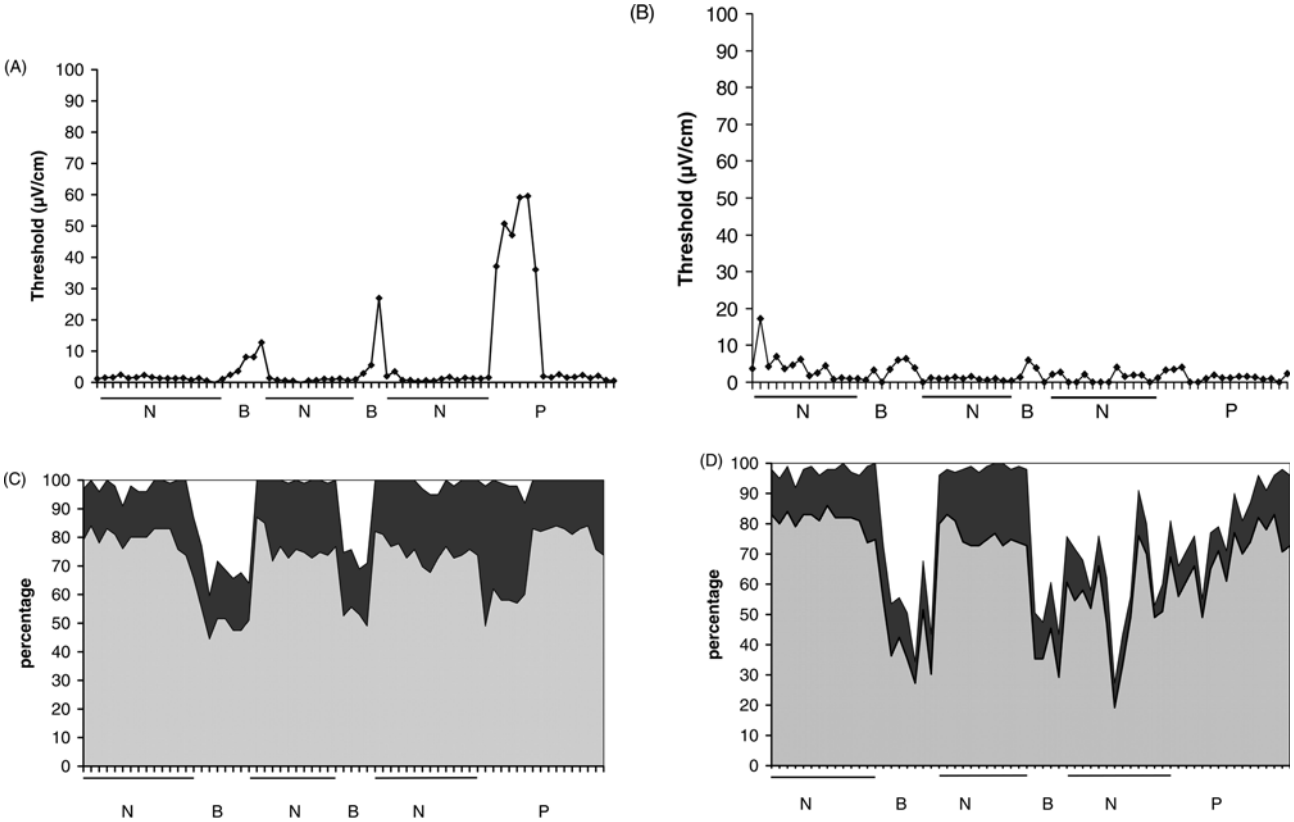

Fig. 2. – The effect of three different types of food on electrodetection performance in catfish. **N**. normal food **B**. food with 0.7% Butylparaben **P**. fish food pellets Trouvit eel 2 mm. (A) Electrodetection thresholds of fish BB. (B) Electrodetection threshold of fish BC. (C) Percentage of correct choices, false choices, and no-go trials of fish BB in a two alternative forced-choice experiment set-up. (D) Percentage of correct choices, false choices, and no-go trials of fish BC in a two alternative forced-choice experiment set-up. In figure C and D, correct choices are represented by the shaded areas, false choices by the black areas and no-go trials by the white areas.

Fish BC showed a similar response pattern when fed with minced beef with Butylparaben (B). An increase in no-go trials and decrease in correct choices were seen ($p << 0.001$, N compared to B) with little influence on the electrodetection threshold ($p = 0.073$, N compared to B). However, after the second set of experiments with food additives, the performances of the fish were never fully restored. Thus, although fish BC was still capable of performing at threshold level, it seemed to have lost its motivation to perform.

DISCUSSION

Since both catfish were capable of performing at a threshold level comparable to that of previous experiments in the same species (e.g. Eeuwes et al., 2001), several conclusions can be drawn from the collected data.

When Butylparaben was added to the food, the threshold level increased in both fish (borderline significance in fish BC). This implies that the fish either had more difficulties perceiving the fields or did not make enough choices to reach its final threshold. If the catfish were experiencing difficulties in perceiving the fields, one would expect an increase of false choices at the cost of correct choices. However, the number of false choices did not increase in either fish ($p = 0.128$ for BB and $p = 0.366$ for BC, N compared to B). Therefore the decreasing percentage of correct choices can be explained by the increase of no-go trials. This indicates that the fish was capable of performing the required task and still had full

control over its electroreceptive system. During testing with Butylparaben, food was not eaten on a regular basis. This fact, in combination with the response behaviour, leads us to conclude that the catfish is not willing to perform when fed with Butylparaben food. Apparently, food with Butylparaben is not considered to be a proper reward by the catfish, and we might even go so far as to state that our fish have 'taste'.

The sudden increase in the electrodetection threshold of fish BB after changing the set-up to one with a food pellet dispenser indicates difficulties in understanding the set-up. One of the main reasons for these difficulties was determined by video-analysis of the experiments; the food pellets floated on the water-surface whereas the fish had been trained to retrieve its reward near the bottom of the tank. Thus, the fish had to relearn where to get its reward. In spite of this and some imperfections of our food distribution device, food pellets proved suitable as a reinforcer or reward in conditioning experiments. Obviously, a fine-tuned system is to be preferred.

The last conclusion that may be drawn is that this kind of experiment is suited for welfare studies. The course of the detection threshold tells us whether or not the fish has control of its sensory system. The number of no-go trials, on the other hand, can be used as a measure to quantify the extent to which the fish dislike the food presented. Since fish generally are willing to work for food, the balance between a fish's appetite and the aversiveness of additives (or other aversive stimuli) gives us a valuable tool for quantifying the aversiveness of a stimulus.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the technical support of Gerard Stooker, Ed van der Veen, Rob Loots, and Rob van Weerden.

REFERENCES

Eeuwes, L.B.M., R.C. Peters, F. Bretschneider & W.J.G. Loos (2001). Electroreception of catfish *Ictalurus nebulosus* in uniform and non-uniform DC fields : detection threshold and body length. *Belg. J. Zool.,* 131 (Suppl. 2) : 73-78

Peters, R.C., W.J.G. Loos, F. Bretschneider & A.B. Baretta (1999). Electroreception in catfish : Patterns from motion. *Belg. J. Zool.,* 129 : 263-268.

Peters, R.C., W.J.G. Loos, F. Bretschneider & P.S. Heijmen (1995a). Psychophysical assessment of catfish electro-sensitivity versus temperature and electric field frequency. *Neth. J. Zool.,* 45 : 3-4.

Peters, R.C., W.J.G. Loos, F. Bretschneider & P. S. Heijmen (1996). Catfish Ictalurus nebulosus (LeSueur, 1819) electrosensory temperature reference curves for aquatic biomonitoring. *Lobon Cervia, J. [Editor]* : 297-300.

Peters, R.C., W.J.G. Loos, M. Hobbelen, F. Bretschneider & J.L.M. Hermens (1995b). Lindane raises catfish electrodetection threshold. *Belg. J. Zool.,* 125 : 185-191.

Peters, R.C. & F. Van Wijland (1974). Electro orientation in the passive electric catfish Ictalurus nebulosus. *J. Comp. Physiol.,* 92 : 273-280.