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THE CYTOSKELETON (1)

by

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SUMMARY

An intricate network of cytoplasmic filamentous structures determines the maintenance of shape and the motility of animal cells. Only some fibers are permanent features, most structural elements of the versatile cytoskeleton assembling and disassembling into building blocks. Tubulin microtubules, actin microfilaments, and the varied intermediate filaments may be ranked in terms of increasing stability of the polymer. Microtubules and microfilaments are intrinsically polarized and possess directional information, that is not shared by intermediate filaments. Tubulin, actin, and the five classes of intermediate filaments each consist of highly conserved molecules composing gene families probably derived from an original gene that was duplicated and modified during evolution. The specific properties of the cytoskeletal fibers according to species or cell type mainly result from their association with binding proteins which meet different requirements, like regulation of polymerization-depolymerization rate, positioning of the cytoskeletal fiber to other homologous or heterologous fibers as well as to plasma or internal membranes, or ATP-driven motor action. In the case of intermediate filaments, the several classes of cell-specific protein subunits are expressed in a developmental and histological pattern. The regulation of cytoskeletal functioning involves ATP or GTP binding, protein phosphorylations and local concentrations in calcium ions.

INTRODUCTION

Fascinating is the generation and maintenance of shape of animal cells, such as neurons with long axons and dendritic arborisation, enterocytes with microvilli, renal podocytes with pedicels, the Protists with pseudopodia or beating cilia. Even more fascinating is the cell motility referring to three basic forms of movement (WARRICK and SPUDICH, 1987). One is the migration of a cell across a substratum. The second is the variety of changes in shape, for example the furrowing that

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occurs when the cell divides. The third is the directed movement of organelles within the cell.

Both specific cell shape and cell motility involve a complex set of protein fibers : the cytoskeleton. This static term appears inappropriate if one refers to multicellular organisms, in which various organ systems participate in functions exerted by the cytoskeleton at the cell level. The cytoskeletal fibers and associated proteins would better correspond to cytobones, cytomuscles, cytonerves, and cytovessels within a cell.

Eukaryote cells contain three major classes of cytoskeletal fibers : 7 nm actin microfilaments, 24 nm microtubules, and 10 nm intermediate filaments. Some microtubules and microfilaments are permanent features of cells, as in flagella, cilia, or the contractile apparatus of muscle. These ordered systems allowed the acquisition of a great deal of information about the molecular structure and function of the cell cytoskeleton. However, I shall focus mainly on unspecialized forms which can be found in a generalized cell.

The Microtubules

Unlike the rigid image portrayed in electron micrographs, most microtubules are dynamic structures capable of being rapidly assembled and disassembled to adjust to an ever-changing cytoplasm.

Microtubules (Fig. 1) are linear polymers of tubulin dimers. Each dimer is a 100 kDa complex of closely related and highly conserved α- and β-tubulin polypeptides. Despite the multiplicity of genes encoding these tubulins in most eukaryotes, all are functionally equivalent. The 24 nm diameter cylindrical wall of the microtubule is formed by protofilaments of tubulin dimers, cytoplasmic and mitotic microtubules typically having 13 protofilaments. The head to tail arrangement of repeating αβ dimers in the tubulin lattice is polarized, giving the microtubule an intrinsic *structural polarity*. The two ends, (+) and (−) are not equivalent. They have specific structural and assembly characteristics and orient the direction of microtubule-associated transport. This is a crucial point.

The *dynamic instability* model (MITCHISON and KIRSCHNER, 1984, CASSIMERIS *et al.*, 1988) is the most accurate interpretation of the dynamic behavior of microtubules assembled from pure tubulin in vitro. According to this model, the ends of growing and shrinking microtubules are different. Each dimeric tubulin contains two bound GTP molecules. Microtubules grow preferentially by addition of GTP-bound tubulin to the (+) end of a preexisting nucleated microtubule. One GTP is then hydrolyzed to GDP during or just after incorporation of the dimer onto the elongating end of the microtubule. If the resulting end of the microtubule has a GDP cap, it is unstable and depolarizes rapidly. Conversely, if additional GTP-bound tubulin adds to the end before hydrolysis of GTP, that end is not only stable but continues to grow. This dynamic unstability is very sensitive to conditions at the ends of microtubules. A high concentration of free tubulin would favor continued growth, and a low concentration would allow a GDP cap to form, causing

depolymerization. Another condition affecting elongation or shrinkage is the rate of hydrolysis of GTP, the regulating factors remaining unknown.

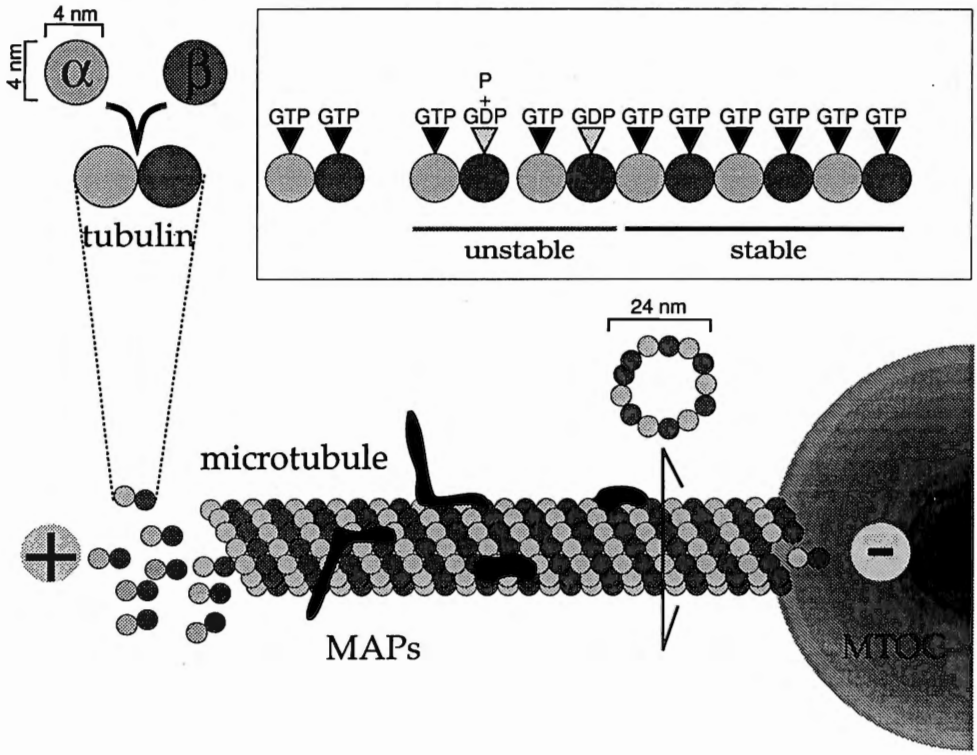

Fig. 1. — Organization of the tubulin microtubule, with microtubule-associated proteins (MAPs) and Microtubule-Organizing Center (MTOC). A scheme of the dynamic instability model is presented in the inset.

Microtubules are not arranged randomly in cells but are organized around one or more discrete foci named *Microtubule Organizing Centers* or MTOCs (see BRINKLEY, 1985). They are specific structural entities with varying forms and distribution, the major one being the centrosome. Due to unknown local conditions, these centers are preferred sites for the initiation, assembly, anchorage and stabilization of microtubules and associated proteins. As demonstrated by applying substances like colcemid and observing the progressive recovery of microtubules from soluble tubulin, these structural templates organize microtubules with defined polarity and distribution. Consequently, the expression of cell form-controlling microtubule patterns appears to involve the MTOCs, and these in turn appear to be controlled by signals arising from endogeneous and exogeneous sources.

Since all tubulins of the multigene family are functionally equivalent, the specific properties of microtubules are then based upon the presence of *Microtubule-Asso-*

ciated proteins or MAPs, a collection of varied molecules that have been defined on the basis of their binding and/or putative interaction with microtubules. In the absence of well-defined functions for most of the MAPs known to date, they are usually classified according to their size as determined by electrophoresis. A first group comprises proteins of very high Mr (>250 000) of which MAP1, MAP2 and dynein are the major components. Next is found a very heterogeneous group of MAPs having a Mr close to 200 000. Other major MAPs include kinesin (Mr 110 000-134 000), STOPs (Mr 72 000-145 000) and chartins (Mr 69 000-80 000). The last set is tau proteins (Mr 55 000-62 000) (see OLMSTED, 1986 ; WICHE, 1989).

If the precise role of most of the MAPs remains speculative, their molecular shape and affinity for other cell components may suggest at least three kinds of functions. First, the smaller MAPs would serve to stabilize, or destabilize, individual microtubules by binding to adjacent tubulin molecules. Second, the larger MAP1 and MAP2 have been visualized at the ultrastructural level as filamentous arms extending from the surface of microtubules. The region protruding from the microtubule may have binding affinities for other microtubules, intermediate filaments, actin microfilaments, serving then as linkers between the other two major cytoskeletal systems. A third function exerted by kinesin- and dynein-like molecules has been recently demonstrated experimentally. Allen Video-Enhanced Contrast microscopy (ALLEN, 1987) can visualize individual microtubules gliding in vitro on the surface of glass slides, or show the bidirectional movement of vesicular organelles along a single microtubule. The addition of MAPs to purified tubulin in this system featured the existence of microtubule-based motors having ATPase activity (MCINTOSH and PORTER, 1989). *Kinesin*-like molecules (300-600 kDa) contain several polypeptides (VALE, 1987). One end of the kinesin molecule can bind to a microtubule, and the other end, to a receptor inserted for example in the membrane of a vesicle. In the presence of ATP, the kinesin molecule moves along the microtubule from the (−) to the (+) end, transporting the bound vesicle with it. In the specific case of nerve cells, this would correspond to anterograde motility for an axonal organelle. On the other hand, the cytoplasmic *dynein* (or MAP 1C, about 350 kDa) is similar in structure and function to dynein in flagella. This large molecule is also composed of several polypeptides. The two globular heads appear to translocate along microtubules in the presence of ATP, and the other end can bind to a vesicle (VALE, 1987, PASCHAL and VALLEE, 1987, SCHNAPP and REESE, 1989). Contrary to kinesin, dynein moves from the (+) to the (−) end of the microtubule, generating then for example retrograde axonal transport. The identification of such molecules provides an answer to one of the older questions in cell biology : how things move from one part of a cell to another ? What is not yet clear is the specificity of the process (DARNEL *et al.*, 1990). How do organelles know whether to bind a protein like kinesin, jump onto a microtubule, and then move ? Some organelles, like pigment granules in melanophores, can alternate their direction of movement along microtubules. They must then contain both receptors for centripetal and centrifugal motors, but only one is active at time. The directionality of movement could be affected by phosphorylation of these receptors or proteins associated with the motor proteins themselves.

The Microfilaments

Several genes code for actin molecules. At least six have been identified in higher vertebrates. Four α-actins are found in muscle cells : one unique to striated muscle, one to cardiac muscle, one to vascular smooth muscle, and one to enteric smooth muscle. Two others called β and γ-actins are found in the cytoplasm of all cells. Clearly, actin genes have evolved from a common precursor and most of the sequence is highly conserved. Regardless of source and species, all actins form the same type of polymer and can copolymerize with all other actins. They probably differ in their affinities for actin-binding proteins and other proteins, although this has not been demonstrated experimentally.

Globular G-actin (42 kDa) is a dumbbell-shaped molecule with dimensions of 6.7, 4, 4 nm (Fig. 2). Microfilaments of F-actin are polymers where a single chain of G-actin monomers forms a helical filament, as deduced from computerized image reconstruction (BULLITT *et al.*, 1988). Since each actin subunit has a defined polarity and the subunits polymerize head to tail, the filaments also have a defined (+)(−) polarity (DARNELL *et al.*, 1990).

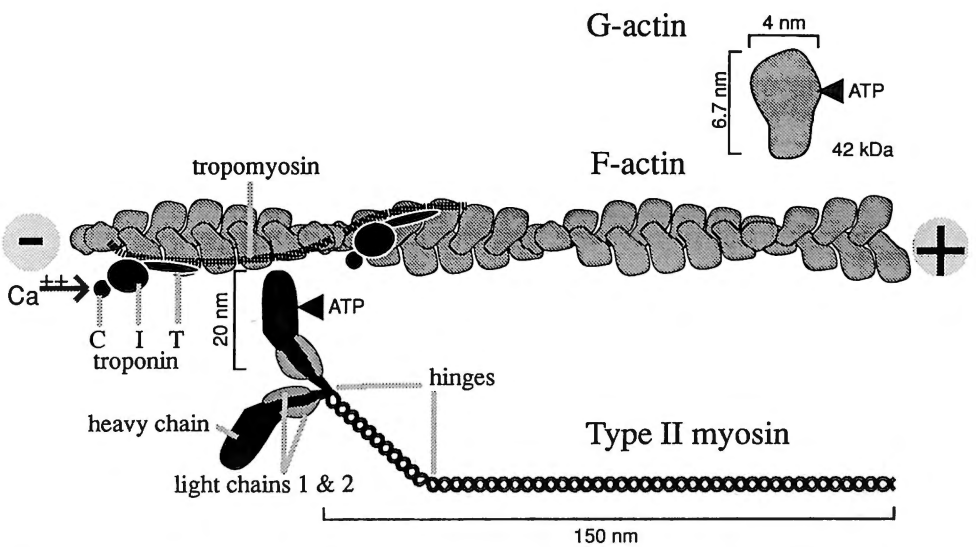

Fig. 2. — Organization of the actin microfilament, and its interaction with type I myosin, as found in the striated muscle cell.

Like microtubules, microfilaments grow by addition of actin subunits to both ends, the rate of growth being 5-10 times faster at the (+) than at the (−) ends. In addition, G-actin molecules contain a special site for ATP or ADP binding. An ATP-actin adds much faster to the microfilament than an ADP-actin. Upon polymerization, the ATP is hydrolyzed, and the resulting ADP remains bound to the F-actin. This hydrolysis does not seem essential for polymerization to occur, but

ADP would have to be displaced by ATP before the structure can be depolymerized (KORN *et al.*, 1987).

Like microtubules again, actin filaments are in equilibrium with monomers, under steady-state conditions. However, two important differences emerge when the assembly-disassembly kinetics are compared between microfilaments and microtubules. First, the polymerization of G-actin is induced by Mg^{2+} and by K^+ and Na^+ at concentrations normally found in the cytosol. In consequence, the dissociation rate of subunits from actin filaments is slow ; they are more stable than microtubules. Second, the critical concentration for assembly of actin monomers into microfilaments is very low, so that the vast bulk of actin should be polymerized within cells (DARNELL *et al.*, 1990).

Since all actins are equivalent, the specific properties of microfilaments according to cell type or cell function result from actin-binding proteins, as was the case for MAPs and microtubules. These actin-binding proteins are quite varied.

The most extensively studied *type II* myosin has been identified in muscle and non-muscle cells (WARRICK and SPUDICH, 1987, SPUDICH, 1989). It is a hexamer composed of one pair of heavy chains (230 kDa) each consisting of a N-terminal globular head 20 nm long, which binds ATP and actin, and a long coiled-coil α helical tail of about 150 nm, which is involved in thick filament formation. Associated to each head are two different light chains, of about 20 kDa. There are two, perhaps three, flexible joints in the molecule. In muscle cells, the hydrophobic tails of 300-400 myosins pack together to form a bipolar aggregate, the thick filament 15-20 nm in diameter and from 1.4 to more than 5 μm long. We shall not consider in detail this specific arrangement, but only recall the interaction of actomyosin.

In addition to actin, the thin filament in striated muscle contains two protein complexes. *Tropomyosin* is a coiled-coil of two α-helical polypeptides, 41 nm long, extending over seven actin subunits. It is bound to actin helix and lies in the groove of the microfilament. Tropomyosin also possesses a specific site for binding another protein complex : *troponin*. Calcium can bind to the C-subunit of troponin, inducing a conformational change to the I-subunit that is bound to actin, and then to the T-subunit bound to tropomyosin. Tropomyosin shifts from the groove in actin filament and exposes a region of the actin monomers to which the myosin heads can bind. Myosin energized by ATPase activity pivots the head to 45°, causing actin to move.

Vertebrate smooth muscle and invertebrate muscle contain tropomyosin, but are devoid of the troponin complex. One of the two light-chain pairs associated with the myosin heads inhibits the interaction of these heads with actin microfilaments. Binding of calcium to the light chain releases this inhibition and activates myosin ATPase activity. Calcium regulation of contraction may also involve the stimulation of activity of a kinase, which phosphorylates one pair of light chains, triggers assembly of bipolar myosin thick filaments and myosin-actin binding (SELLERS and ADELSTEIN, 1987).

The role of phosphorylations at multiple sites of the non-muscle Type II myosin remains unclear at present. The C-terminal portion (the tail) of the heavy chain can

be phosphorylated, which appears to decrease the ability of the myosins to form filaments and lowers their actin-activated ATPase activity. The regulatory myosin light chain can be reversibly phosphorylated as well, by a calcium-calmodulin-dependent myosin light chain kinase, which allows actomyosin interaction. These phosphorylations seem to make non-muscle myosin versatile motors which can adapt to the changing requirement of non-muscle cells, as for exocytosis, cytokinesis, cytoplasmic streaming (CITI and KENDRICK-JONES, 1987, KORN and HAMMER, 1988).

Type I myosin or minimyosin is a 110 kDa protein that has one actin-binding head and a short tail that binds to phospholipids in membranes. It exists namely in filopodia and microvilli, where it might determine retraction or positioning of the microfilaments. It is also thought to be able to move vesicles along actin (WARRICK and SPUDICH, 1987).

A large number of actin-binding proteins (Fig. 3) are known in addition to the myosin motors described hereabove (POLLARD and COOPER, 1986).

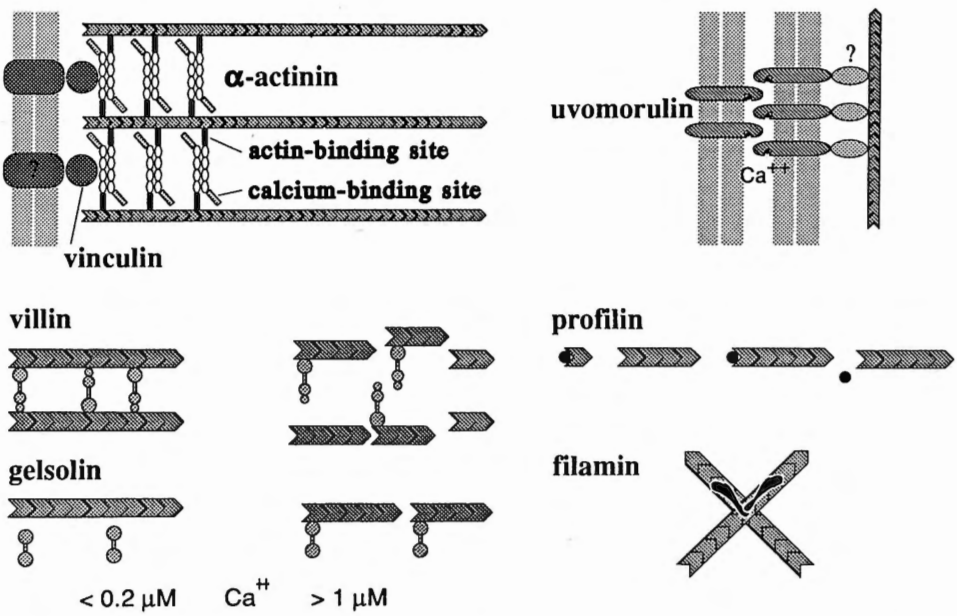

Fig. 3. — Some actin-binding proteins (modified from DARNELL *et al.*, 1990). See text for comments.

Some serve as cross-linking and attachment proteins for actin filaments, such as *α-actinin, dystrophin and spectrin.* They form antiparallel dimers or tetramers, where the N-terminal domains form the actin-binding site, and the C-terminal domains contain calcium binding sites. These two sections are highly conserved. The middle sections consist of repeats of triple helical segments. The antiparallel feature of

these proteins exposes actin binding sites at each extremity of the molecule, which both can bind actin filaments.

With the help of other proteins, the attachment proteins may also participate in anchoring actin filaments to membranes. At intercalated disks in cardiac muscle, or dense plaques of smooth muscles, *vinculin* is thought to bind to an integral membrane protein and to α-actinin, which cross-links actin filaments and attaches them to the membrane. Similarly, in epithelial cells, the trans-membrane *uvomorulin* (or E-cadherin) is concentrated in the junctional region called the belt desmosome, where microfilaments encircle the cell under the plasma membrane. Uvomorulin links the plasma membranes of adjacent cells together in a calcium-dependent manner, and on the other hand, anchors the sides of actin filaments to the membrane via a set of actin-binding proteins that are not yet clearly identified.

In brush-border cells, in addition to *minimyosin* linked by the tail to the plasma membrane and positioning or retracting bundles of microfilaments of the microvilli, other actin-binding proteins are found. In the core of the microvillus, *fimbrin* (68 kDa, 1 molecule per 10 actin monomers), tightly packs parallel actin bundles. *Fodrin,* a long protein similar to spectrin in its structure and function, links adjacent actin bundles, as well as actin to integral proteins in the plasma membrane. The cross-linking *villin,* found in the core has a dual function as being also a regulator of actin filament stability. Villin (95 kDa) contains three domains : both N- and C-termini can bind actin, serving then as cross-link at low calcium concentration (< 0.2 μM), but at high calcium concentration (> 1 μM), the conformational change of this protein gives severing properties towards microfilaments. The N-terminus of villin remains bound to the (+) end of each actin fragment at the severed site.

Gelsolin is another protein causing disassembly of actin networks. It ressembles villin, but has only one actin binding site at the N-terminal domain. Thus, it cannot cross-link filaments. It is only an actin-severing protein, which binds to actin at micromolar concentrations of calcium, cuts the filament, and remains attached to the (+) end of the fragment. In the same line of thinking, *profilin* (15 kDa) can bind to actin monomers forming profilactin, that can attach only to the (+) end of a filament. However, the (+) end of many existing filaments is already capped by other proteins (cap Z, α-actinin, villin, gelsolin, ...). Profilin decreases then the polymerization rate, and if all filaments are capped, blocks all actin polymerization.

A last actin-binding protein to be evoked is *filamin* (270 kDa), a stabilizing one. This long (160 nm) and flexible molecule possess two actin-binding sites near its ends. Associated dimers of filamin can connect actin filaments by forming crossed branches, causing then the formation of a three dimensional network.

The Intermediate Filaments

The third class of cytoplasmic fibers are the 8 to 12 nm diameter intermediate filaments (IF, Fig. 4). Since some cell types appear not to contain IF, it seems unlikely that IF have fundamentally important « housekeeping » functions, but it is probable that they are involved in specialized functions related to the differentiation

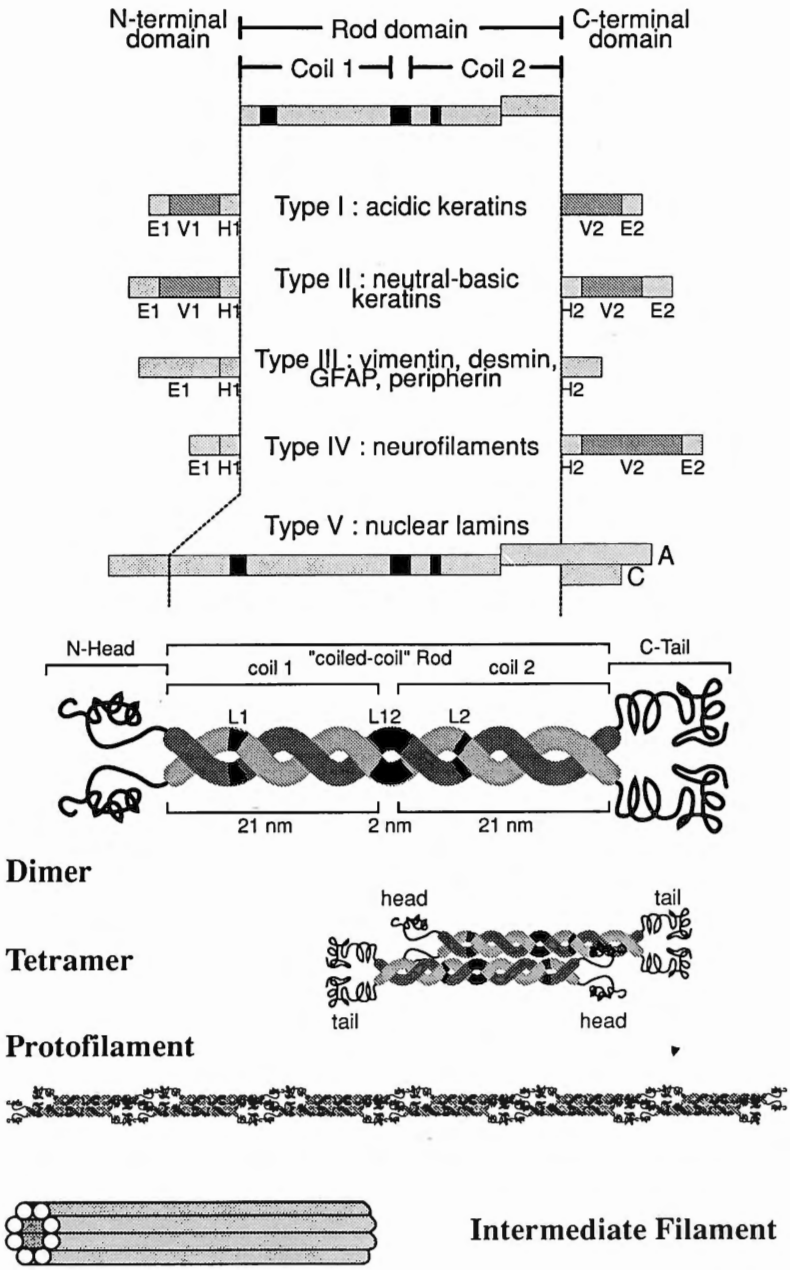

Fig. 4. — Organization of the intermediate filaments (IF) and the chemical classification of IF and nuclear lamins (modified from STEINERT and ROOP, 1988). E : end sequences ; V : variable sequences ; H : homologous sequences ; L1, L2, L12 : linkers. A scheme of IF polymerization is also presented.

state of the cell, including mechanical coordination of the cytoskeleton, information transport, and signal transduction (STEINERT and ROOP, 1988). IF proteins constitute an extremely heterogeneous multigene family (30 or more per mammalian species), being then far more complex than other major classes of cytoskeletal fibers, actin microfilaments and tubulin microtubules. IF proteins are expressed in a developmental and histological pattern. Although the typical differentiated cell expresses only one type of IF, coexpression has been repeatedly described, and it occurs also during development, as cells switch from one IF type to another and some cells seem to remain stuck at a point of coexpression (OSBORN and WEBER, 1983, TORELLI *et al.*, 1989).

The *vimentin* gene is expressed typically in mesenchymal cells such as fibroblasts or endothelium. The fibers often terminate at the nuclear envelope and at the plasma membrane, suggesting the continuity of the IF network in cells by connecting the plasma membrane cytoskeleton to the nuclear lamina or karyoskeleton. They may also function to keep organelles like lipid droplets in a defined place within the cell. The *desmin* gene is expressed predominantly in myogenic cells. The fibers can be arranged like vimentin IF, and also determine the specific arrangement of the contractile apparatus of actomyosin. The Glial fibrillary acidic protein (*GFAP*) is the product of one gene expressed in glial cells and astrocytes. These three first types of monomers, of about 50 kDa, form homopolymeric filaments.

In contrast, neurons express at least three different proteins of increasing molecular weight, which copolymerize into neurofilaments. They are named *NF-L* (60-70 kDa), *NF-M* (105-110 kDa), and *NF-H* (135-150 kDa). A fourth gene has been identified more recently, which is expressed in neurons of the peripheral nervous system, hence the name *peripherin* (57 kDa). These neurofilaments complexed to microtubules formed in the cell body move down the axon allowing strength and rigidity.

More complicated are the cytokeratins expressed in epithelial cells. Some 30 of these are divided into two classes : types I (acidic, 40-60 kDa) and II (neutral/basic, 50-70 kDa). Both type I and type II keratins are required for 10-nm filament formation at the heterodimer level ; they are obligate copolymers. Each type of epithelium has its characteristic complement of multiple cytokeratins, found in tightly packed bundles known as tonofilaments distributed throughout the cytoplasm and forming an elaborate cage around the nucleus. They are associated with desmosomal plaques in regions of cell-cell contacts.

Recently, a new type of IF has been added to the list, the lamins forming the nuclear skeleton on the inner nuclear surface of the nuclear membrane. The fibers are arranged in a quasi-tetragonal meshlike lattice, and consist of at least four proteins (60-75 kDa) in vertebrates.

Amino acid sequences show all intermediate filament peptides to contain a highly conserved central rod domain, approximately 40-nm long. This domain exhibits repeated heptad of amino acids with hydrophobic residues in positions a and d. These heptad repeats are typical of a coiled-coil configuration between two molecules. The rod is interrupted by three non-conforming helical regions, the spacers.

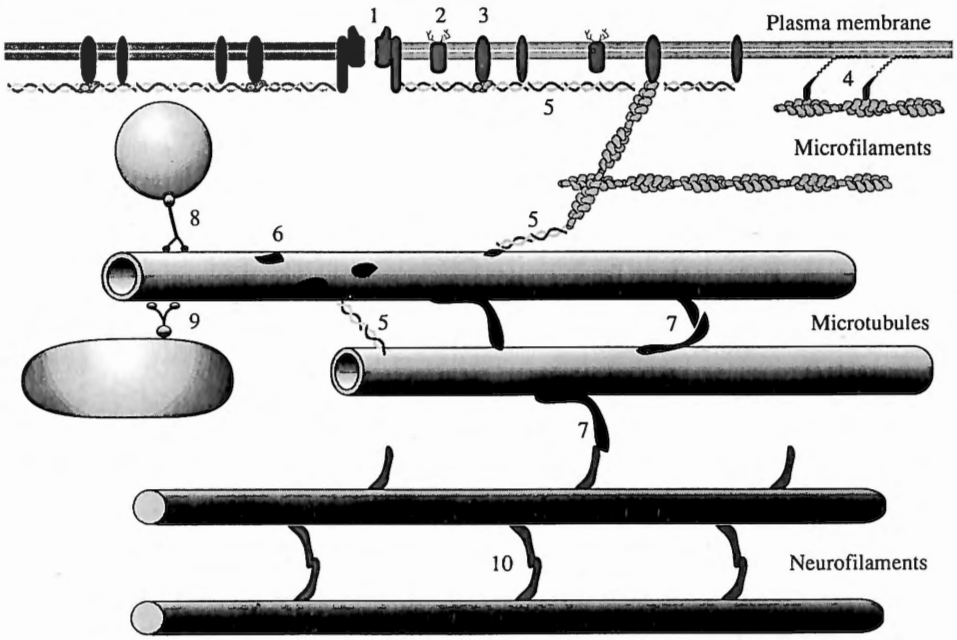

Fig. 5. — Schematic representation of interactions between cytoskeletal components in mature neurons (modified from RIEDERER, 1990). The plasma membrane contains ion channels (1), integral membrane glycoproteins (2) and anchoring proteins like ankyrin (3). Type I myosin (4) is also associated with the membrane. Spectrin (5) forms a submembrane network anchored on ankyrin, coats microtubules, may form crossbridges between microtubules, microfilaments, and is involved in the interaction between microtubules and microfilaments. Microtubules are stabilized by MAP tau (6) and are cross-linked via MAP 2 (7). Microtubules are also tracks for anterograde and retrograde transport of membraneous organelles by kinesin (8) and dynein (9). Intermediate filaments are cross-linked by extensions of the IF molecules or IF-associated proteins (10).

At the ends of the central rod domain are a N-terminal head and a C-terminal tail that vary between the individual intermediate filament peptides, and which are responsible for the differences in molecular weight and biochemical properties.

The assembly of the two monomers in the dimer is parallel, but a tetramer is formed by antiparallel, staggered side-by-side aggregation of two dimers, so that it is no longer polarized. This will distinguish IF from both microfilaments and microtubules which each possess a clear structural directionality. Tetramers aggregate end-to-end to form a protofilament, and eight protofilaments form a cylindrical 10 nm thick filament. Unlike what would be expected from stable polymers as are known IF, they are dynamic structures that exchange subunits with a small soluble pool (ANGELIDES *et al.*, 1989 ; MILLER *et al.*, 1991)

There is evidence that the head plays a role in the assembly of filaments by means of phosphorylations, and at least in neurofilaments, phosphorylations of the tail control the cross-linking state and space-filling properties (MATUS, 1988).

A growing list emerges of minor proteins that frequently coisolate and/or associate with the major IF structural proteins, and that are termed IF-associated proteins (IFAPs). It seems possible to assign these IFAPs into different functional classes that may also correlate with their size (STEINERT and ROOP, 1988). (1) IFAPs of low Mr (10-45 kDa) that bind IF laterally into tight macrofilament aggregates, as filaggrin or high-sulfur and high tyrosine-glycine protein families ; (2) IFAPs of high Mr that cross-link IF into loose networks, like paranemin, synemin, plectin, ... ; (3) IFAPs that function as « capping » proteins, like ankyrin, spectrin, desmoplakin, lamin B ; (4) several IFAPs that do not appear to conform to this scheme, such as epinemin and internexins.

Conclusions

The concluding remarks can be illustrated by presenting possible interactions of cytoskeletal elements in an axon (Fig. 5). Most structural elements of the versatile cytoskeleton assemble and disassemble into building blocks. In terms of increasing stability of the polymer, are found tubulin microtubules, actin microfilaments, and the varied intermediate filaments. Each group consists of highly conserved molecules composing gene families probably derived from an original gene in a primordial eukaryotic cell, that was duplicated and modified during evolution. Due to the polarization of the monomer and the head-to-tail polymerization, microfilaments and microtubules are intrinsically polarized with (+) and (−) ends conferring directional information. This is not shared by intermediate filaments. The specific properties of the cytoskeletal fiber according to species or cell type result from two different aspects. In the case of intermediate filaments, several classes of cell-specific protein subunits exist. In the case of microfilaments and microtubules, the widely distributed actin or tubulin molecules can associate with binding proteins. Intermediate filaments also possess associated proteins. These diverse binding proteins meet different requirements. They participate to the regulation of polymerization-depolymerization rate, in addition to GTP or ATP bound to the subunits and the monomer concentration. They ensure positioning of the cytoskeletal fiber to other homologous or heterologous fibers and to plasma or internal membranes. They exert ATP-driven motor action, like type I and II myosins, kinesin, and dynein. Several aspects of cytoskeleton functioning including the association with binding proteins, cross-linking, space-filling properties, and polymerization, are headed up by local concentration in calcium and other ions, or protein phosphorylation.

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THE COLLAGEN FAMILY OF PROTEINS : TWO DISTINCT LINES OF EVOLUTION (1)

by

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SUMMARY

Fourteen well-defined collagen types are known in vertebrates and a few of their counterparts have been described in invertebrates. A single, ancestral collagen type might have been expected in the most primitive multicellular animals, the sponges. These organisms actually contain collagen in two forms, cross-striated fibrils and a large assembly of thin filaments constituting the spongin skeleton. A molecular biology study has revealed that these two forms correspond to two distinct genetic collagen types. Several short-chain collagen sequences have been deduced from their cDNA. They bear homologies with nematode cuticular collagens, with basement membrane collagens and with the type XIII collagen of vertebrates. The corresponding genes are expressed in cells secreting the spongin. A fibrillar collagen has also been characterized. Its structure at the C-terminal non-helical domain and its gene organization allowed a comparison with the vertebrate type XI fibrillar collagen, considered as an axial core contained in vertebrate larger fibrils.

The multicellular animals are not only characterized by their cells, numerous, differentiated and coordinated, but also by their unique intercellular medium, composed of several macromolecular species. This extracellular matrix is involved in the mechanical integration of the different cells and tissues, and, in addition, modulates the cell differentiation and behaviour (TRELSTAD, 1984). Among the various components of the extracellular matrix, collagen is certainly the most universal protein (BAIRATI and GARRONE, 1985).

Its skeletal function has been obviously recognized as collagen is the main organic component of bone and constitutes the framework of cartilage. Collagen is also involved in specialized structures such as fish scales (FRANCILLON, *et al.*, 1990)

(1) Presented at the Second Belgian Congress of Zoology, Diepenbeek, Belgium, 15-16 November 1991.

and in several skeletal devices in invertebrates (BAIRATI and GARRONE, 1985). In the most primitive multicellular animals, the sponges, collagen can form a reticulate organic skeleton, for example the skeleton making the natural bath sponges. In this case, the coarse fibers are composed of the association of thin unit filaments of spongin (about 10 nm in diameter), collagenous in nature. In other species, the deposit of such filaments is used to link together siliceous needles, the spicules, building then a skeleton combining silica and collagen. In addition, these filaments are used to stick the sponges to rocks, shells, or Petri dishes in the laboratory (Pl. I). In all species of sponges, the extracellular matrix contains banded fibrils, 20 nm in diameter (Pl. I), scattered or associated in bundles, which are also composed of collagen (GARRONE, 1978). Similar fibrils are found in the cnidarians, and fibrils with a more complex banding pattern are current constituents of extracellular matrices of almost all other animals.

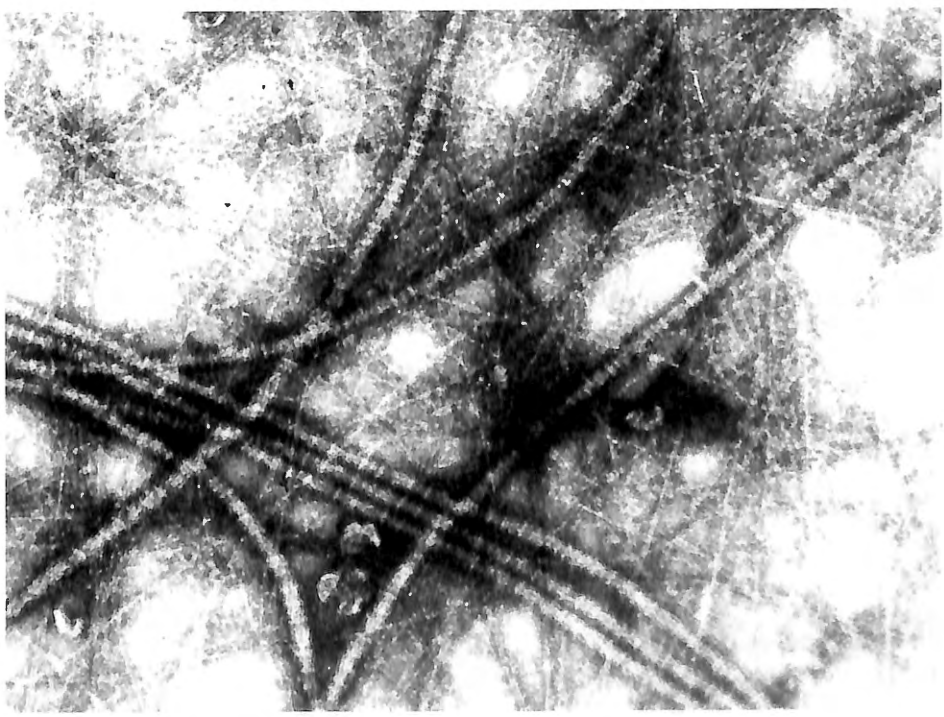

Plate 1. — Electron microscope view of the two collagen structural units present in sponges : collagen fibrils (the largest fibrils) and spongin filaments (the thinnest elements) in the background. Negative staining, X 100,000.

These collagen fibrils are formed by the regular association of collagen molecules. Each molecule itself is a triple helix formed by the intertwining of three helical polypeptides, the alpha chains. The amino acid sequences of the collagen

alpha chains are characterized by the repetition of triplets Gly-Xaa-Yaa. This conformation is stabilized by the presence of imino acids in about 30 % of the Xaa and Yaa positions (prolyl and hydroxyprolyl residues respectively). Actually, the extremities of the molecule are composed of non helical domains (often termed non-collagenous domains) which may or may not be removed during the maturation of the molecule. For some collagens, short, non helical domains can also interrupt the helical domain. Fourteen different collagen types are known in vertebrates (VAN DER REST and GARRONE, 1991). They form a variety of extracellular assemblies : banded fibrils (types I, II, III, V and XI), fibril coats (types IX, XII, perhaps XIII, and XIV) ; sheets (types IV — the basement membranes —, VIII and X), beaded filaments (type VI), short anchoring segments (type VII).

Several hypotheses have been considered to determine the most primitive of these collagen types. In order to bring a contribution to this point, we investigated the collagen of sponges using a molecular biology approach. We choose a freshwater species (*Ephydatia mülleri* LIEB) easy to use in laboratory experiments. From 3-day-old sponges, total RNA populations were isolated and translated *in vitro*. Among the translation products, four peptides were sensitive to collagenase digestion. Two of them had an estimated molecular mass of about 200 and 160 kDa respectively. They can be compared to precursors of vertebrate alpha chains of fibrillar collagens. The two other collagenase-sensitive translated peptides had a molecular mass of 81 and 48 kDa respectively and they correspond to short-chain collagens (EXPOSITO *et al.*, 1990). Poly(A)-rich RNA was then used to construc a cDNA library with λ gt 10 as vector. This library was screened first with a probe corresponding to a human collagen (type I, α1 chain). A clone, EmC4, was isolated and characterized. It coded for a short-chain, non-fibrillar collagen (EXPOSITO *et al.*, 1990). The 1.2kb insert of this clone was then used to screen the same library at high stringency. Several additional positive clones were detected, which all coded for collagenous polypeptides with a structure similar to the previous one, encoded by EmC4 (Fig. 1). These polypeptides contain two helical domains (66 and

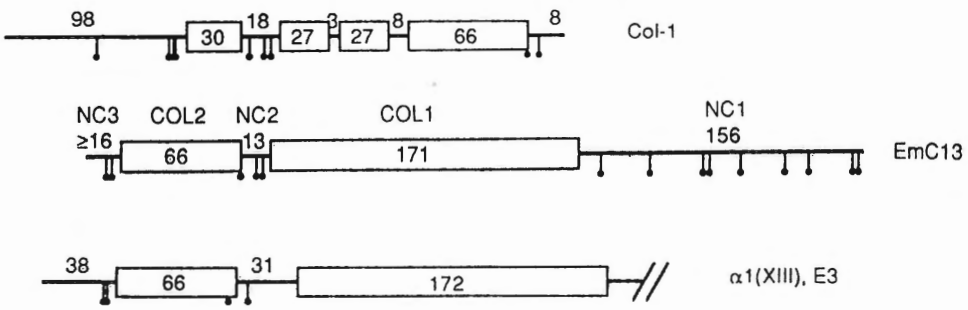

Fig. 1. — Comparison of the structure of a sponge short-chain collagen chain (EmC13), a nematode cuticular chain (Col-1) and a type XIII collagen chain (α 1 XIII, E3). The solid lines correspond to non-helical domains (NC) ; the boxes indicate the helical domains (COL). The numbers correspond to the number of amino acid residues in each domain. The position of cysteines are shown by vertical bars with a dot (modified from EXPOSITO *et al.*, 1991).

171 amino acid residues) separated by a short non-helical domain (13 to 16 amino acid residues, with 2 or 3 cysteines), and a C-terminal, non-helical domain of 156 amino acid residues with 9 cysteines (EXPOSITO *et al.*, 1991). These collagen chains had similarities with various other vertebrate and invertebrate collagen chains. The helical domains are comparable in size to the helical domains COL-1 and COL-2 of vertebrate type XIII collagen (66 and 172 amino acid residues) ; the central, short non-helical domain is similar in size and cysteine positions with interruptions in nematode cuticular collagens (EXPOSITO *et al.*, 1991) ; and the C-terminal non-helical domain can be compared to the same domain of type IV (basement membrane) collagen in vertebrates (EXPOSITO *et al.*, 1990). The genomic DNA of *E. mülleri* was digested with several restriction endonucleases and analyzed by Southern blotting. Using the EmC4 probe, 10 to 12 bands were revealed for each enzyme used, indicating that approximately 10 highly homologous genes belong to the same family. They all encode short chain collagens (EXPOSITO *et al.*, 1991). This situation is reminiscent of the nematode cuticular collagen gene family (COX *et al.*, 1984). By *in situ* hybridization methods, it was demonstrated that the cells expressing these genes were secreting the collagenous filaments (spongin) around spicules and in the basal, sticking cement (EXPOSITO *et al.*, 1991).

Screening of the cDNA library also revealed a clone, C 23, positive only at low stringency, and coding for a different collagen chain named Emf1α. The conceptual translation product comprises an uninterrupted helical domain (with one imperfection due to an additional amino acid insertion in one of the triplets) and a non-helical, C-terminal domain containing 7 cysteines (EXPOSITO and GARRONE, 1990). Computer alignement of this domain with comparable domains of vertebrate and sea urchin fibrillar collagen chains revealed homologies with vertebrate type XI collagen (EXPOSITO and GARRONE, 1990).

A genomic library was then constructed by inserting *E. mülleri* genomic DNA fragments into the *Xho* I site of λ GEM-11 DNA (Promega). This library was screened using the available sponge collagen probes. With the probe corresponding to the fibrillar collagen, the isolated gene encoded the Emf1α collagen chain (EXPOSITO and GARRONE, 1990). All the exons coding for the helical domain begin with a complete Gly codon and terminate with a complete Yaa codon. Moreover, for most of them, their size is 54 bp or a multiple of 54 bp. These two features are typical of the gene organization of fibrillar collagens (Fig. 2). Additional characters lying in the 3' end of the regions coding for the helical domain strenghten the comparison with type XI collagen (EXPOSITO and GARRONE, 1990). This comparison is highly significant if one considers that in vertebrates the type XI collagen (and also type V) has been postulated as forming a thin axial core inside large collagen fibrils (MENDLER *et al.*, 1989). Using the probes corresponding to the short-chain collagens, the genes which were cloned had not the characteristic organization of fibrillar collagen genes. The exons coding for the helical domains had variable sizes and they all began with split Gly codons (EXPOSITO *et al.*, 1991).

In conclusion, sponges contain at least two collagen types (Pl. I). One is a fibrillar collagen, presumably forming the cross-striated fibrils present in the intercellular spaces of all species. The small diameter of these fibrils (20 nm) and their

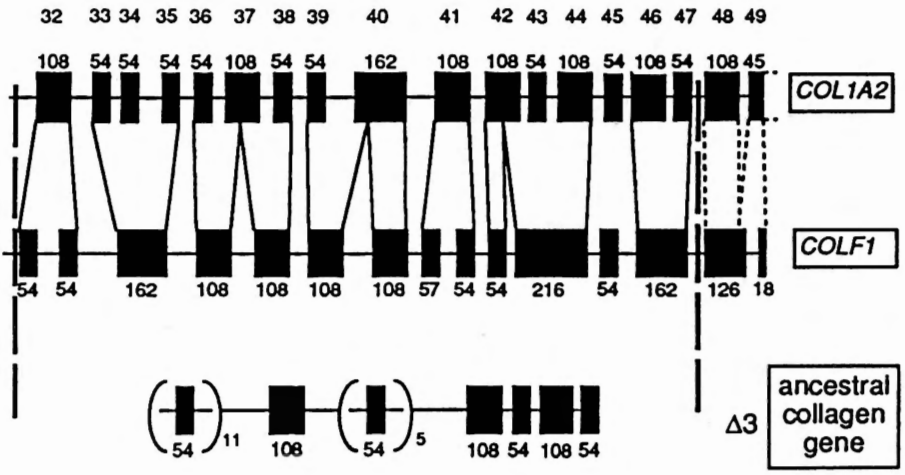

Fig. 2. — Comparison the organization of vertebrate and sponge fibrillar collagen genes for the region coding for the C-terminal end of the helical domain. The black boxes indicate the exons. *COL1A2* : gene coding for the chain α 2 of the type I collagen ; *COLF1* : sponge fibrillar collagen gene. The upper line indicates the vertebrate exon numbers. The numbers above (verbrate) and below (sponge) the exons indicate their size in base pairs. In the bottom of the figure is represented a tentative reconstruction of an hypothetical ancestral collagen gene.

very simplified banding pattern make them rudimentary collagen fibrils. It is reasonable to think that their counterpart in vertebrates (and probably in more organized invertebrates) are the fibrillar collagen types V and XI supposed to constitute the internal core of large collagen fibrils. The second sponge collagen type might be present as a basal cement in most species and as a skeletal component in certain groups. It is clearly the main constituant of the thin spongin filaments. This collagen has in fact a surface localization, even if it is secondarily internalized to make the organic skeleton. It is then not surprizing if it has features reminiscent of nematode cuticular collagens. As it is also deposited by cell layers, the comparison with basement membrane collagens is explainable. This collagen might then be the percursor of (1) external, secreted collagens such as some skeletal collagen in cnidarians, cuticular collagens in nematodes and annelids, byssus threads in molluscs, egg case in selachians, and (2) internal basement membrane collagens (BAIRATI and GARRONE, 1985). These two collagen types have probably envolved in completely separate ways.

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OPTIMIZATION OF SKELETAL STRUCTURE IN VERTEBRATES (*)

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SUMMARY

Stresses due to bending moments generally predominate in long bones. They can be equalized along the length of the bone shaft if the bone tapers so as to make section modulus proportional to distance from the distal end. For marrow-filled bones, there is an optimum ratio of radius to wall thickness that minimizes mass for given strength, but different strength criteria give different optimum ratios. Increasing the strength of a bone reduces the probability of failure but increase the cost of growing the bone and the energy cost of moving it. A theory of optimum safety factors has been formulated but has not been used quantitatively because of the difficulty of expressing the cost of failure in the same currency as the other costs. A theory of optimum elastic stiffness successfully predicts the thicknesses of typical tendons. The possibility that the stiffness of bones is optimized, rather than their strength, is considered.

INTRODUCTION

Bones should be strong enough to withstand the forces that will act on them in life. They should not be unduly heavy, for a heavy bone (especially if it is a limb bone) is cumbersome : it may increase the energy cost of running or reduce the animal's maximum speed. How should bones be constructed, to be as light as possible for their strength ? What is the best compromise between strength and lightness ? We can expect evolution to optimize the proportions and thickness of bones whether it does so directly, by means of genes that specify bone dimensions, or indirectly, by genes that control the bone growth that occurs in response to the forces that the bone experiences (Lanyon, 1981).

The forces on the distal end of a bone can be resolved into axial and transverse components. The axial component sets up compressive stresses that are uniform

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across any cross-section. The transverse component, however, tends to bend the bone and sets up stresses that range from a maximum compressive stress at one face to a maximum tensile stress at the other. Experiments in which bone stresses in running or jumping have been calculated from force plate records, or from the output of surgically implanted strain gauges, show for most of the long bones of limbs that transverse forces (that exert bending moments) are much more important generators of stress than are the axial forces (BIEWENER *et al.*, 1983, report an exception). Accordingly, in the following discussion, we will take account only of the bending moments that bones have to withstand.

BONE SHAPE

Long bones are generally tubular, with shafts that taper towards the distal end. How, precisely, should they be shaped ?

The bending moment acting on a cross-section of a bone that is in equilibrium can be calculated by taking account only of forces on one side of the section : for example, distal to it. Muscle attachments are commonly restricted to the proximal ends of long bones, so for much of the length of the bone the bending moments can be calculated by taking account only of the transverse force on the distal end, and are proportional to the distance of the section from the distal end. The maximum stress due to a bending moment in a cross section can be calculatrd by dividing the bending moment by the section modulus, a geometric property of the cross section (see ALEXANDER, 1983b). A structure is only as strong as its weakest part so, to be as strong as possible for their weight, the shafts of bones should taper so

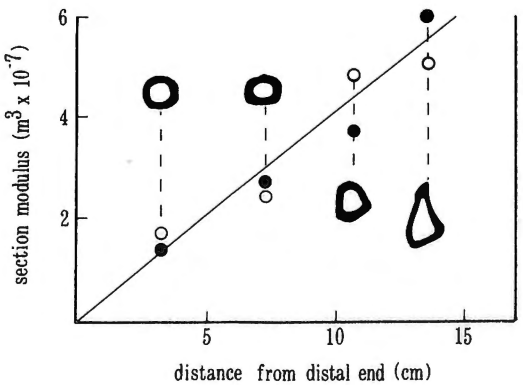

Fig. 1. — A graph of section modulus (both moduli are shown) against distance from the distal end of a dog tibia. The measured sections are illustrated with the anterior edge uppermost. From ALEXANDER (1975).

as to have section moduli proportional to distance from the distal end. Fig. 1 shows that this is approximately the case for the tibia of a dog, but ALEXANDER and VERNON (1975) found less good agreement in the case of a kangaroo tibia.

The strength of a bone in bending is proportional to the section modulus, and the weight per unit length to the cross-sectional area. The ratio of section modulus to cross-sectional area is greater for hollow tubes than for solid rods, so tubular structure gives strength with lightness. Accordingly, most long bones are tubular like the example of Fig. 1.

The ratio of the radius R of a tubular bone to the thickness t of its wall can have any value between one and infinity. What is the optimum? For a bone of given strength, as R/t increases (as the bone becomes a thinner-walled tube) the mass of the bone itself decreases but the mass of marrow within it increases. PAUWELS (1980) realized that this implied that there must be an optimum value of R/t, but CURREY and ALEXANDER (1985) pointed out a complication : the optimum value depends on how strength is defined. Fig. 2 shows that if bones of equal ultimate strength or impact strength are compared, the optimum value of R/t is 2.2. However, for bones of equal yield strength or fatigue strength the optimum is 3.0 and for bones of equal stiffness it is 4.0.

CURREY and ALEXANDER (1985) measured R/t for 228 long bones of 56 species. For terrestrial mammals they found values ranging from 1.0 to 3.7, with a marked mode at about 2. This is consistent with the hypotheses that bones are optimized for impact strength or for ultimate strength. As might be expected, much higher

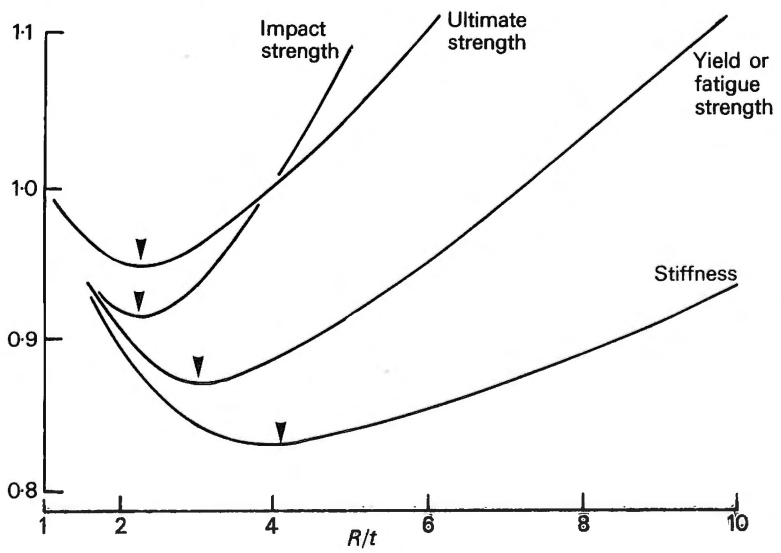

Fig. 2. — Masses of tubular marrow-filled bones (relative to the mass of a solid bone of equal length and strength) plotted against the ratio of radius to wall thickness. Curves are shown for several different strength criteria. From CURREY and ALEXANDER (1985).

values of R/T are common among such bird bones as are filled with gas instead of marrow.

FACTORS OF SAFETY

We have discussed how bones should taper, and what proportions their tubular cross sections should have. Next we ask, how strong should bones be?

The factor of safety of an engineering structure is the ratio of the strength it is designed to have, to the maximum load it is expected to have to take. Engineers

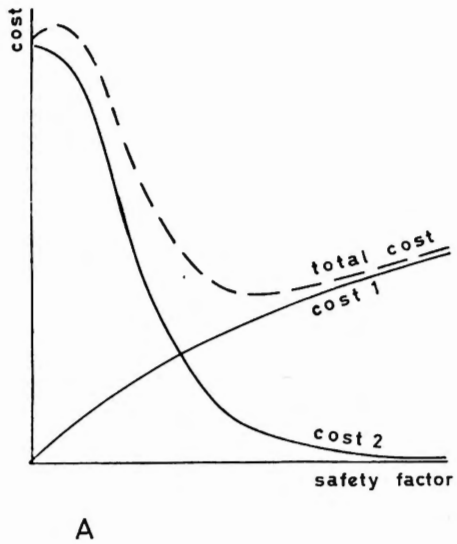

Fig. 3A. — Graphs of the costs associated with bones of different safety factors, according to the theory described in the text.

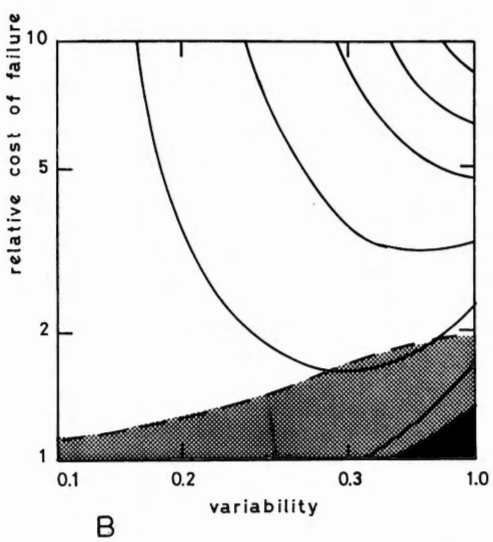

Fig. 3B. — A graph of the cost of failure against the variability of maximum loads, with contours showing the safety factor that minimizes total cost. Stippling indicates that the minimum is only a local one (the global minimum is at zero safety factor) and the black area indicates that the only minimum is at zero safety factor.

generally design structures with factors of safety much larger than one. This is necessary, to reduce the probability of failure to an acceptable level, because there is variation in actual strength, between structures built to the same design, and because maximum loads cannot be predicted precisely. Measurements of the stresses in bones in strenuous activities of various animals show that long bones commonly have factors of safety between 2 and 5 (ALEXANDER, 1981 ; BIEWENER *et al.*, 1983). They nevertheless break : frequencies of healed fractures in long bones

range from about 0.005 in birds to 0.03 in human (Amerindian) populations (BRANDWOOD *et al.*, 1986). Symmetry of strength between left and right bones of a pair in birds indicates that variability of strength is not a severe problem : bones need factors of safety principally on account of the unpredictability of the loads they have to bear (ALEXANDER *et al.*, 1984).

ALEXANDER (1981) presented a theory of optimum safety factors. I identified two costs that vary with safety factor (Fig. 3A). One, the cost of growth and use of the bone, increases with increasing safety factor : stronger bones require more energy and material for their growth and (because they are heavier) more energy for limb movements. The second cost, which decreases with inceasing safety factor, can be thought of as the notional cost of an insurance policy against failure : it is the probability P that failure will occur, multiplied by the cost of a failure. ALEXANDER (1981) gave reasons for modelling the cost of growth and use as proportional to (safety factor)$^{2/3}$ and the probability 1-P that the bone will *not* fail as a cumulative lognormal function of the safety factor.

This model has two parameters : the cost of failure (expressed as a multiple of the cost of growth and use for a safety factor of one) and the variability of maximum load (expressed as the standard deviation of the lognormal function). For many combinations of these parameters, the total cost has a minimum value for some non-zero safety factor (Fig. 3A). However, if the cost of failure is too low, the optimum safety factor is zero, and the bone can be expected to disappear in the course of evolution (Fig. 3B).

This model is regrettably difficult to apply quantitatively, because the costs of failure and of growth and use are generally most easily measured in different currencies (mortality and energy, respectively), and the exchange rate is unknown. However, some insight is given by the qualitative prediction that safety factors will be high when the cost of failure is high, especially if the variability of maximum load is also high (Fig. 3B). For example, the immensely thick leg bones of some moas (Dinornithes) may be due to the lack of any threat from predators, so that these herbivorous, flightless birds had little need to run. The cost of use for heavy leg bones was therefore low, making the relative cost of failure high (ALEXANDER, 1983a).

The theory of optimum safety factors was extended by ALEXANDER (1984) to take account of the danger of fatigue fracture.

OPTIMUM STIFFNES

To perform their functions, bones must be stiff as well as strong. A discussion of optimum stiffness for them is best introduced by an account of the theory of tendon stiffness which inspired it.

Some tendons serve as springs to save energy in running (ALEXANDER, 1988). Their elastic compliance is essential to this function, but the compliance of other tendons brings a disadvantage. Consider a muscle whose function is to develop a force and shorten, so as to move a joint through some required angle. If its tendon

stretches, the muscle must shorten more to move the joint through the same angle. A muscle that has to shorten more needs longer muscle fibres (fibres with more sarcomeres in series) if it is to work only within the range of lengths at which it is capable of exerting large forces. If a tendon is made thinner (and so lighter), it will stretch more in use, and will require a muscle with longer fibres (which will therefore be heavier, for the same physiological cross-sectional area). Using this approach, KER *et al.* (1988) argued that the total mass of muscle plus tendon would be least if the cross-sectional area of the tendon was about 1/34 of the physiological cross-sectional area of the muscle. The stress in the tendon, when the muscle exerted its maximum isometric force, would then be about 10 MPa, only one tenth of the tensile strength of tendon. A survey of tendons in the legs and tails of various mammals showed that for the great majority of them, the ratio of tendon and muscle areas was close to this theoretical optimum. Tendons that serve as springs in running, however, are much more highly stressed. CUTTS *et al.* (1991) found that the thickness of tendons in a human forearm were close to the theoretical optimum.

ALEXANDER *et al.* (1990) applied the same reasoning to long bones. A slender bone is light but is also relatively flexible. Because it bends, muscles must shorten more to bring the distal end of the limb to a required position. To be capable of shortening more, the muscle requires longer fibres, so must be heavier. Reasonable assumptions about the properties of bone and muscle led to the conclusion that the total mass of bone plus muscle could be least, if the thickness of the bone were such that peak stresses of $\pm$ 70 MPa acted in each cross-section, when the muscles exerted their maximum isometric force.

Peak stresses of around $\pm$ 70 MPa are indeed commonly found in limb bones, in strenuous activities (ALEXANDER *et al.*, 1990). It would however be wrong to conclude at this stage that bone stiffness rather than bone strength is optimized. Such stresses may well result from selection for optimum safety factor : they correspond to a safety factor of about three.

CONCLUSION

The examples in this paper show how plausible models predict optimum proportions for bones. We should not ask simply whether a bone is strong enough for its function, or whether it is as light as possible. Rather, we should ask whether its dimensions are optimal.

Unfortunately, the predictions of the models are ambiguous. The optimization model for tubular proportions predicts different ratios of radius to wall thickness, depending on how strength is defined (Fig. 2). It has not yet been possible to apply the theory of optimum safety factors quantitatively, because exchange rates between currencies are unknown. The theory of optimum stiffness leads to a clear prediction, but the agreement between observed and predicted stresses may be coincidental.

The compositon of bones may vary, as well as their dimensions, leading to differences in mechanical properties (CURREY, 1984). A complete theory of bone design would seek to optimize composition as well as dimensions.

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(Manuscript received on 31 October 1991)

REDESCRIPTION OF *ACANTHOCHONDRIA ATELEOPI* CAPART, 1959 (COPEPODA : POECILOSTOMATOIDA) PARASITIC ON THE DEMERSAL FISH, *ATELEOPUS LOPPEI* FROM THE EASTERN GULF OF MEXICO

by

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ABSTRACT

Acanthochondria ateleopi CAPART, 1959 is redescribed from two female and two male specimens. The male is described for the first time. *Acanthochondria ateleopi* is characterized by, and distinguished from its congeners, by an elongate, cylindrical, and distinctly segmented first antenna. This is the first report of this copepod from the western Atlantic.

Key Words : parasitic copepod, *Acanthochondria ateleopi,* redescription.

INTRODUCTION

CAPART (1959) described a new species of parasitic copepod from the demersal fish, *Ateleopus banardi,* POLL, 1953 collected off the west coast of Africa. This new species, *Acanthochondria ateleopi* was not described in sufficient detail. Only habitus figures and brief descriptions of the appendages were included in the original work. CAPART (1959) did, however, mention a few features which serve to distinguish *A. ateleopi* from its congeners : shape of the body and structure of the antenna. The original description was based on only one adult female (a male was also found but not described). During a recent examination of the branchial chambers of *Ateleopus loppei,* ROULE, 1922 collected off Florida, 4 specimens of *A. ateleopi* (2 females, 2 males) were found. In this paper *A. ateleopi* is redescribed in detail based on this new material.

MATERIAL AND METHODS

The host *Ateleopus loppei* (1011 mm, total length) was collected at a depth of 307 m off the west Florida coast on 6 February, 1989. (The host specimen is catalogued (GSBC No. 85376) as *Ijimaia loppei* in the family Ateleopidae). This family is currently under study by K. J. SULAK. SULAK (pers. comm.) indicates that *Ijimaia* is a junior synonym of *Ateleopus,* and *A. loppei* is probably synonymous with *A. barbardi,* from which CAPART's specimens of *Acanthochondria ateleopi* were obtained). The host was fixed whole at sea in 10 % formalin and later transferred to 50 % isopropanol. The parasites were recovered from the branchial chambers and retained in isopropanol. One adult female, with attached male was retained intact, the other female and male was dissected. Appendages from these specimens were removed and mounted in Turtox CMC-S stain-mountant or 85 % lactic acid. Line drawings were made with the aid of a camera lucida (dissecting scope) or drawing tube (compound scope) at magnifications up to 900X. Terminology of morphological structures follows that of KABATA (1979).

Acanthochondria ateleopi Capart, 1959
(Fig. 1 and 2)

Host : Ateleopus loppei (University of Florida, Cat. No. 85376)
Site of Infection : branchial chamber (dorsal portion)
Locality : off southwest coast of Florida, Gulf of Mexico (26°25'N-84°45'W)
Specimens : two adult females, two adult males ; Atlantic Reference Centre, St. Andrews, New Brunswick

Redescription (based on four specimens)

Adult female : Cephalosome (Fig. 1, 1a-1 b) about as wide as long, posterior lateral margin protruding, rounded. Dorsal surface (Fig. 1, 1a) with medial bisecting ridge, raised slightly from surface. Oral area (Fig. 1, 1b) on ventral posterior surface of cephalosome. Trunk with anterior neck mass formed from first two thoracic leg-bearing segments ; dorsal posterior margin of each with shall, transverse wrinkles. Trunk many times larger than cephalosome with roughly parallel lateral margins ; pronounced transverse indentations in body wall at mid-length. Posterolateral processes distinct, rounded at ends and curving inwards toward genito-abdomen. Genito-abdomen (Fig. 1, 2) at central posterior margin of trunk ; small, curving ventrad. Genital segment (Fig. 1, 2) with medial groove on dorsal surface and striated lacunae on either side. Abdomen (Fig. 1, 2) clavate, with uropods at mid-length on lateral margins. Uropods (Fig. 1, 3) with swollen base and single seta at apex. Distal part divided into two portion ; basal portion clavate, with two large setae at tip (on either side of terminus), terminal portion tapering to digitiform tip, surface armed with fine spinules. Total length of parasites 7.8 and

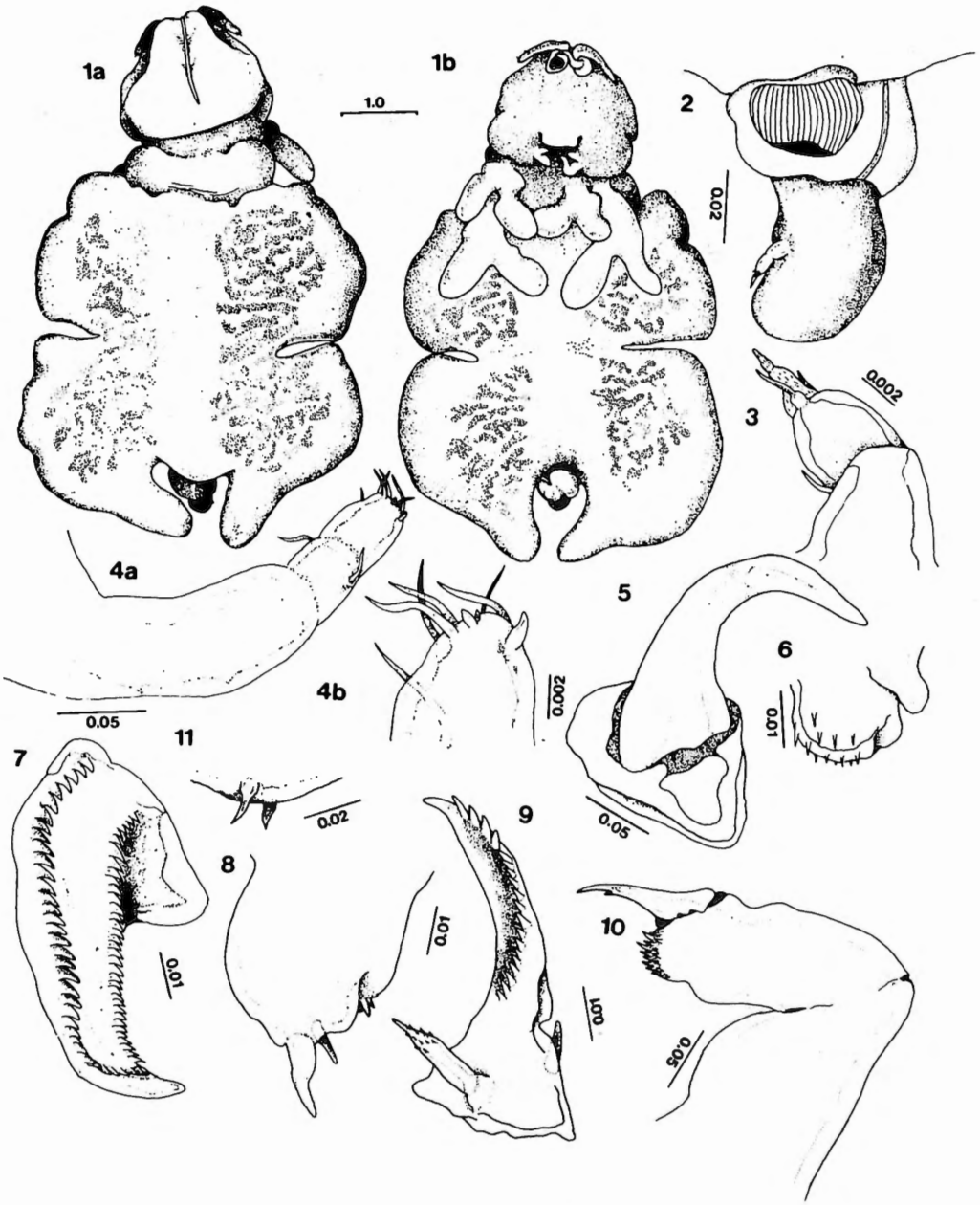

Fig. 1. — *Acanthocondria ateleopi* Capart, 1959, adult female ; 1a : habitus, dorsal ; 1b : habitus, ventral ; 2 : genito-abdominal complex, dorsolateral ; 3 : uropod, lateral ; 4 : first antenna ; a. entire appendage, dorsal, b. apical armature, dorsal ; 5 : second antenna, ventral ; 6 : paragnath, ventral ; 7 : Mandible, dorsal ; 8 : first :axilla, ventral ; 9 : claw of second maxilla, dorsolateral ; 10 : maxilliped, ventral ; 11 : tip of endopod of second leg, ventral.

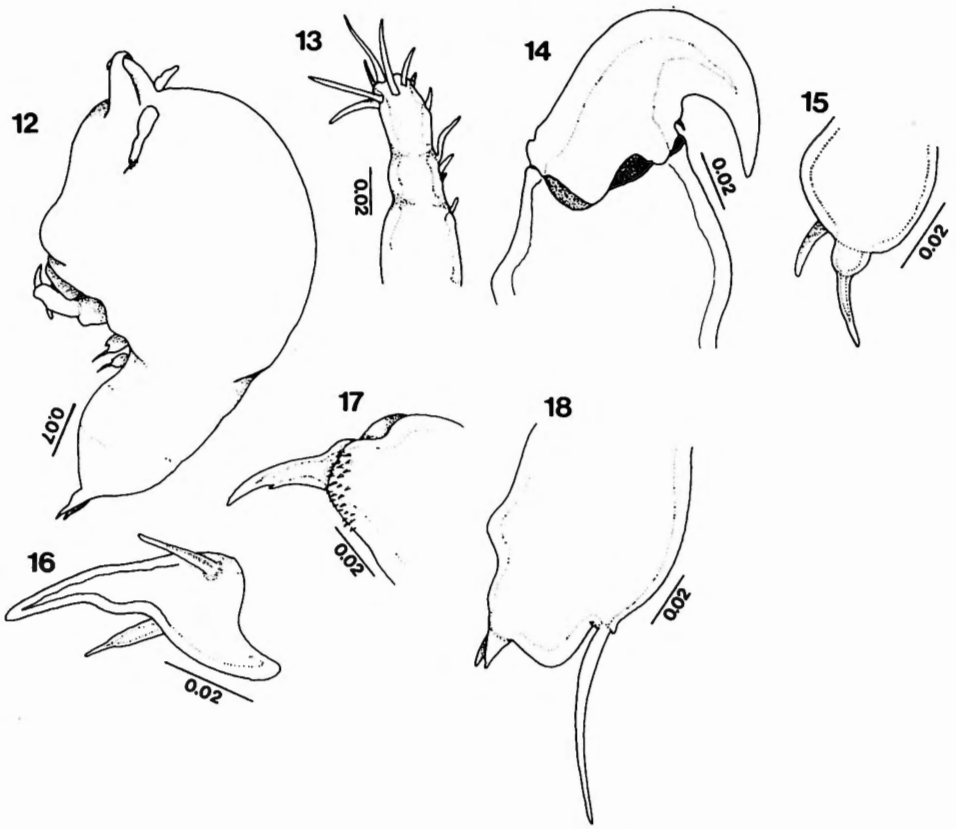

Fig. 2. — *Acanthocondria ateleopi* Capart, 1959. 12 : adult male, lateral ;13 : first antenna, lateral ; 14 : second antenna, ventral ; 15 : first maxilla, ventral ; 16 : claw of second maxilla, ventral ; 17 : claw and brachium of maxilliped, ventral ; 18 : first thoracic leg, lateral. (Scale bars are in millimeters).

8.0 mm, respectively. First antenna (Fig. 1, 4a) elongate, cylindrical ; distinctly three-segmented, basal segment pedunculate, comprising two-thirds length of appendage, unarmed. Penultimate segment shorter, cylindrical, with two spiniform setae on dorsal surface. Terminal segment slightly longer than penultimate, cylindrical ; apical armature (Fig. 1, 4b) consisting of eight setae : five spiniform, three much shorter, papilliform. Second antenna (Fig. 1, 5) with sub-quadrangular, flattened basal plate and elongate, recurved distal hook Paragnath (Fig. 1, 6) with rounded end and distinct lateral process ; end armed with minute denticles on ventral surface. Labrum (Fig. 1, 1b) truncate, flap-like, with rounded posteriolateral corners, no discernable armature. Mandible (Fig. 1, 7) falcate, curved inward at tip ; with 35 teeth on outer margin and 34 teeth on inner margin. First maxilla (Fig. 1, 8) globular with one large and one small seta at terminal end, two small spines and cleft on medial surface. Second maxilla with slightly curved claw (Fig. 1,

8), armed with 19 teeth on outer margin; auxiliary process with tapering tip, provided with several spinules; single blunt seta on dorsal surface of second maxilla at base. Maxilliped (Fig. 1, 10) with large thorn-like spines on bracjium; claw with secondary tooth on inner margin, near tip. First thoracic leg (see Fig. 1, 1b) bilobate, ends rounded, unarmed. Second thoracic leg (Fig. 1, 1b) twice size of first, bilobate with rounded ends. Terminus of endopod armed with two sub-triangular setae (Fig. 1, 11).

Adult male : cephalosome (Fig. 2, 12) globular, inflated. Posterior trunk conical, pseudosegmented. Uropods like those of female, but lacking large seta at base. First antenna (Fig. 2, 12) three-segmented; basal segment broad, cylindrical, comprising one-half length of appendage; armed on anterolateral corner with one short, spiniform seta. Penultimate segment cylindrical, one-half length of first, provided with three spiniform setae on anterior lateral margin. Terminal segment slightly longer than second, armed with eight spiniform setae of various lengths. Second antenna (Fig. 2, 12) with short, stout base; claw (terminal segment) with broad base and stout recurved tip, short seta at base on posterior inner margin. Mandible not clearly observable, similar in shape to that of female. First maxilla (Fig. 2, 15)globular, armed with one large terminal seta and one shorter, sub-terminal seta. Claw of second maxilla (Fig. 2, 16) with expanded base and tapering tip; two auxiliary processes on either side at base. Maxilliped (Fig. 2, 17) similar to that of female, but with proportionally smaller spines on brachium. First thoracic leg (Fig. 2, 18) with two short triangular setae on posterior inner corner and one long, flagelliform seta on other outer corner. Second leg similar to first, slightly smaller.

DISCUSSION

Given the identity of the host, the shape of the body and the morphology of the appendages, there is little doubt that the specimens described herein belong to *A. ateleopi* as described by CAPART (1959). *Acanthochondria ateleopi* is unique among its congeners in possessing an elongate, cylindrical, and segmented first antenna. CAPART (1959) described his specimen from *Ateleopus* as having long and narrow first antennae. Within *Acanthochondria* OAKLEY, 1930 (50 nominal species), the predominant form for this appendage is short and subcircular with little or no descernable segmentation. *Acanthochondria ateleopi* most closely resembles *A. cornuta* (MULLER, 1776), a parasite of flatfishes in the north Atlantic, noted for its morphological plasticity (the specimens described herein resemble most closely the cornuta-form (c.f. HO. 1970). However, *A. ateleopi* exhibits a trunk and neck which is broader, longer and relatively more massive than that characteristic of *A. cornuta,* and a mandible with fewer teeth. KABATA (1984) considered the shape of the first antenna to be useful in distinguishing species of *Acanthochondria*; given the structure of this appendage, (belonging in the type A group (see KABATA, 1984) : digitiform and simple, broadest at base, tapering gradually to tip), *A. ateleopi* can be considered a valid species. This is the first report of this parasite from the west Atlantic, and the first since the original description.

ACKNOWLEDGEMENTS

I thank Dr. Ju-shey Ho for advice on the validity of *A. ateleopi,* Dr. Mark Leiby (Flor. Mar. Res. Instit.) for providing the host specimen, Dr. K. J. Sulak for advice on host nomenclature, Dr. Z. Kabata for reviewing a draft of this paper and Cheryl Winchester for typing the manuscript.

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METIS REDUCTA n. sp. and *LAUBIERIA TERCERA* n. sp. (HARPACTICOIDA, METIDAE) FROM THE SOUTHERN COAST OF PAPUA NEW GUINEA*

by

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SUMMARY

Metis reducta n. sp. and *Laubieria tercera* n. sp. (Harpacticoida, Metidae) collected off Motupore Island (southern coast of Papua New Guinea) are described. The description of a male specimen of *L. tercera* n. sp. allows the extension of the generic diagnosis. A key to the three known species of the genus *Laubieria* SOYER is presented.

Key-words : Metidae, *Metis reducta, Laubieria tercera,* male characteristics.

INTRODUCTION

The harpacticoid family Metidae assembles two genera : *Metis* PHILIPPI, 1843 and *Laubieria* SOYER, 1966 unifying five and two species, respectively. Recently, MIELKE (1989) discussed at length the problems in species discrimination within the genus *Metis*. The poor quality of original descriptions and various erroneous identifications in the past have made three species out of five difficult to recognize. However, the reduced segmentation of the P1 of the herein described *M. reducta* n. sp., distinguishes it clearly from all other known members of the genus.

The two species of the genus *Laubieria* SOYER, 1966 presently (*L. corallicola* SOYER, 1966 and *L. secunda* WELLS, 1967) were described on the basis of female specimens only. The discovery of a male of *L. tercera* n. sp. allows for extending the generic diagnosis. A key to the species of *Lauberia* is given to facilitate future determinations.

MATERIAL AND METHODS

The sample yielding the here described animals was collected by scraping of the upper layer of the sediments and was fixed in the field with a buffered formaldehyde solution. The animals were stored in 75 % neutralized ethylalcohol after being picked out. The dissected parts were mounted in glycerine, the coverglass sealed with nail polish. Slides are deposited in the Invertebrate collections of the « Koninklijk Belgisch Instituut voor Natuurwetenschappen ».

Drawings were made with the aid of a camera lucida, on a light microscope equipped with phase contrast. Terminology and abbrevations are according to LANG (1965) and MIELKE (1989).

SYSTEMATICS

genus *Laubieria* SOYER, 1966

Diagnosis — Metidae ; genital somite fused ventrally only ; copulatory pore situated near the fusion line of the somites ; rostrum large, blunt with slender sensillae ; antennule six-segmented with aesthetascs on third and ultimate segments ; exopodite of antenna obsolete, represented as a spiniform seta ; mandible with cylindrical palp ; maxillule with arthrite having sharply bent spines and a slender seta ; coxa, basis and rami obsolete, represented as a minute palp bearing a single seta ; maxilla with endites represented as setae ; basis extended ; maxillipeds not fused, one-segmented, each bearing three setae ; rami of P1 — P4 three-segmented lacking outer spines on one or two segments ; both fifth legs fused, forming a single median plate, bearing one seta and one spine on both sides ; without an exopodal segment.

Sexual dimorphism : antennule sub-chirocer, six-segmented and bearing aesthetascs on third, fourth and ultimate segments ; P5 with longer inner spines ; P6 symmetrical.

The genus comprises : *Laubieria corallicola* SOYER, 1966 (type-species), *L. secunda* WELLS, 1967 and *L. tercera* n. sp.

Key to the species

1. — Third exopodal segments P3 and P4 with 5 setae/spines, in all ; first exopodal segments P3 and P4 without outer spine : 2
   — Third exopodal segment P3 and P4 with 6 setae/spines, in all ; first exopodal segments P3 and P4 with outer spine : *L. corallicola*
2. — Median exopodal segment P2 without inner seta ; inner spine of female P5 short, about 20 % of the outer seta : *L. tercera*
   — Median exopodal segment P2 with inner seta ; inner spine of female P5 long, about 75 % of the outer seta : *L. secunda*

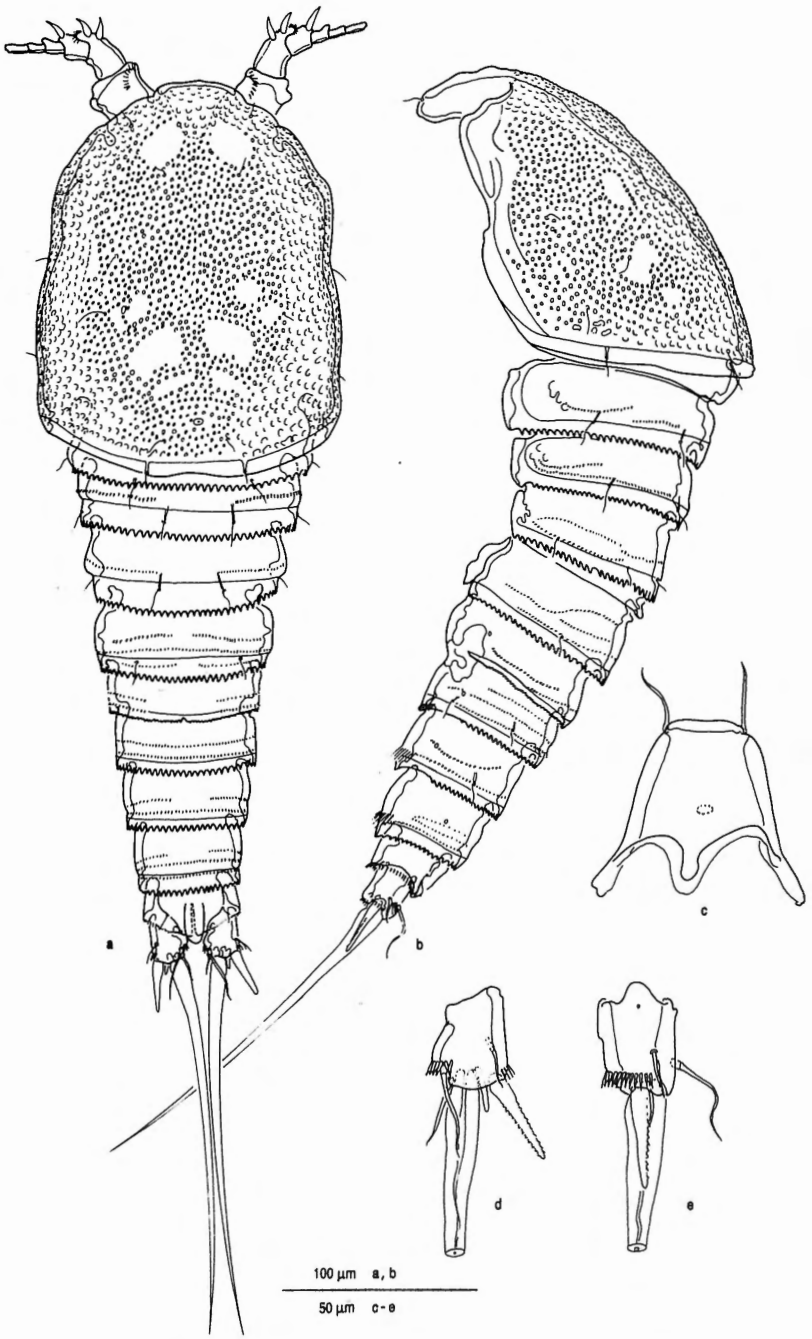

Fig. 1. — *Laubieria tercera* n. sp. : a, habitus of the female, dorsal view ; b, idem, lateral view ; c, rostrum, ventral view ; d, right furcal ramus, dorsal view ; e, left furcal ramus, lateral view.

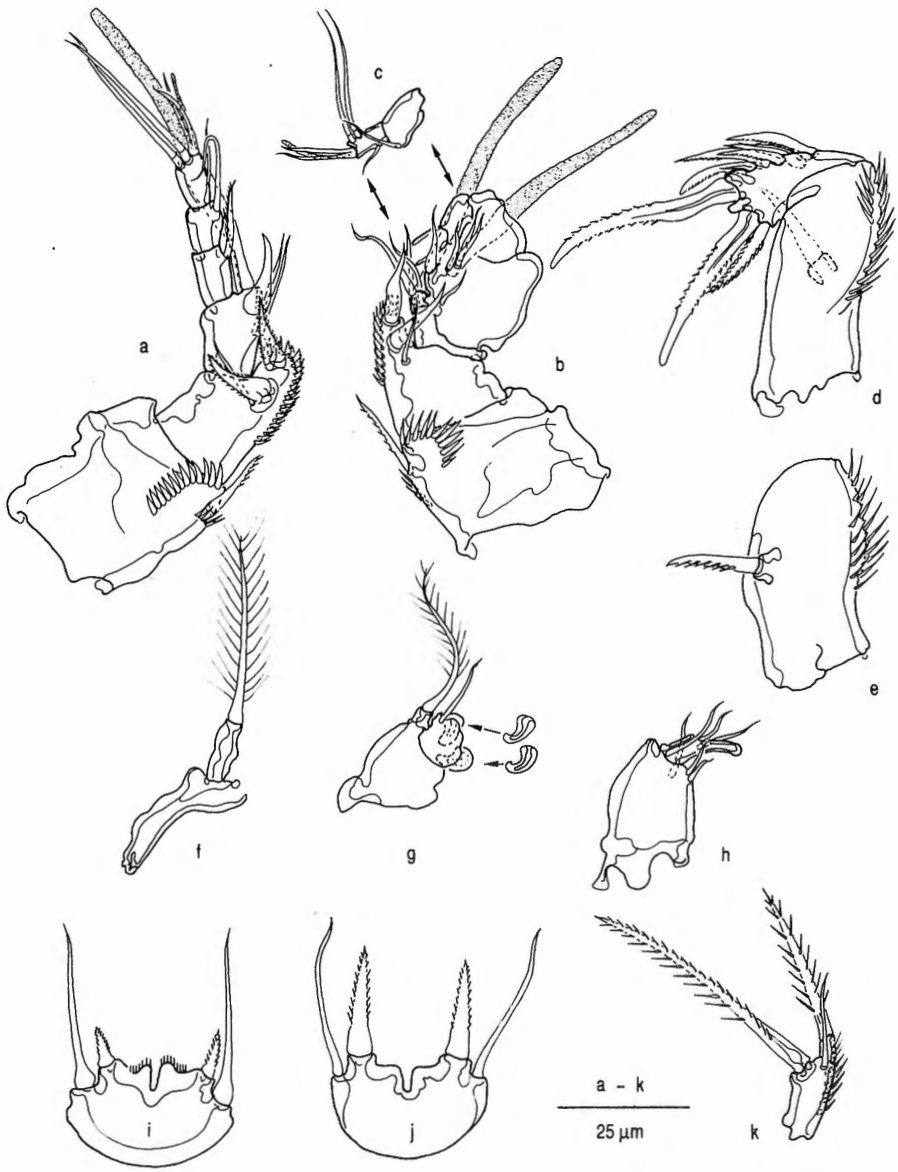

Fig. 2. — *Laubieria tercera* n. sp. : a, female antennule ; b, male antennule ; c, ultimate segments of male antennule ; d, antenna, inner view ; e, allobasis of antenna, outer view ; f, mandible ; g, maxillule ; h, maxilla ; i, female P5 ; j, male P5 ; k, maxilliped.

Laubieria tercera n. sp.

Type material. Holotype, one female dissected on two slides, labeled COP 3411 A and B ; allotype, one dissected male, mounted on two slides, labeled COP 3418 A and B.

Type locality. Papua New Guinea, Capital District, Motupore Island. Upper layer of sediments at − 1 m (during high tide) near the northern end of the island (east of the, locally called, « sand spit »). Leg. F. Fiers, November 22, 1986, field no. PNG 86-85.

Etymology. Lauberia tercera n. sp. is the third species described in the genus. The specific name refers to it.

Description

Female : habitus (Fig. 1a and b) typically *Metis*-shaped with obviously large cephalothorax and bent in lateral view ; length, including furcal rami, 460 µm ; cephalothorax, in dorsal view, slightly shorter than half the body length, with nearly parallel lateral margins, curved towards the rostrum in the anterior quater ; lateral margins of body somites tapering towards the anal one ; genital double-somite fused ventrally only.

Integumental structures : surface of the cephalothorax densely pitted, except for a symmetrical pattern of smooth dorsal areas ; posterior margin of the cephalothorax smooth ; integument of the other somites furnished with few rows of minute spinules dorsally and laterally ; postero-dorsal and lateral margins of the somites with an incised hyaline frill ; ventral surface of the genital and abdominal somites with few rows of minute spinules and with a median row of long spinules near the posterior margin of the second somite ; hyaline frills along the ventral margins of the abdominal somites more slender than dorsally, unincised ; ventral surface of the anal somite with a deep triangular sinus (Fig. 4a) ; anal operculum narrow, rounded distally and smooth.

Furcal rami (Fig. 1d and e) about 1.5 times as long as wide ; outer margin straight, the inner one distinctly convex in the second half ; proximal lateral seta, arising beyond the middle of the ramus and somewhat dorso-laterally implanted ; distal lateral seta spiniform, about as long as the ramus and arising from the outer distal edge ; dorsal seta, articulating on a single basal part and implanted close to the inner distal edge ; outer principal seta highly reduced and blunt ; inner principal seta long, smooth and considerably thickened in the proximal parts of the stem ; inner distal seta slender, not as long as the supporting ramus.
Rostrum (Fig. 1c) large, ventrally directed and fused with the cephalothorax ; lateral margins slightly concave ; distal margin with two sensillae and straight.

Antennule (Fig. 2a) six-segmented, with principal aesthetasc arising from the third segment ; first segment robust, having a comb of large spinules on the dorsal surface and a single seta ; second segment strongly extended distally into a rounded process, furnished with strong spinules anteriorly and bearing two armed spines and

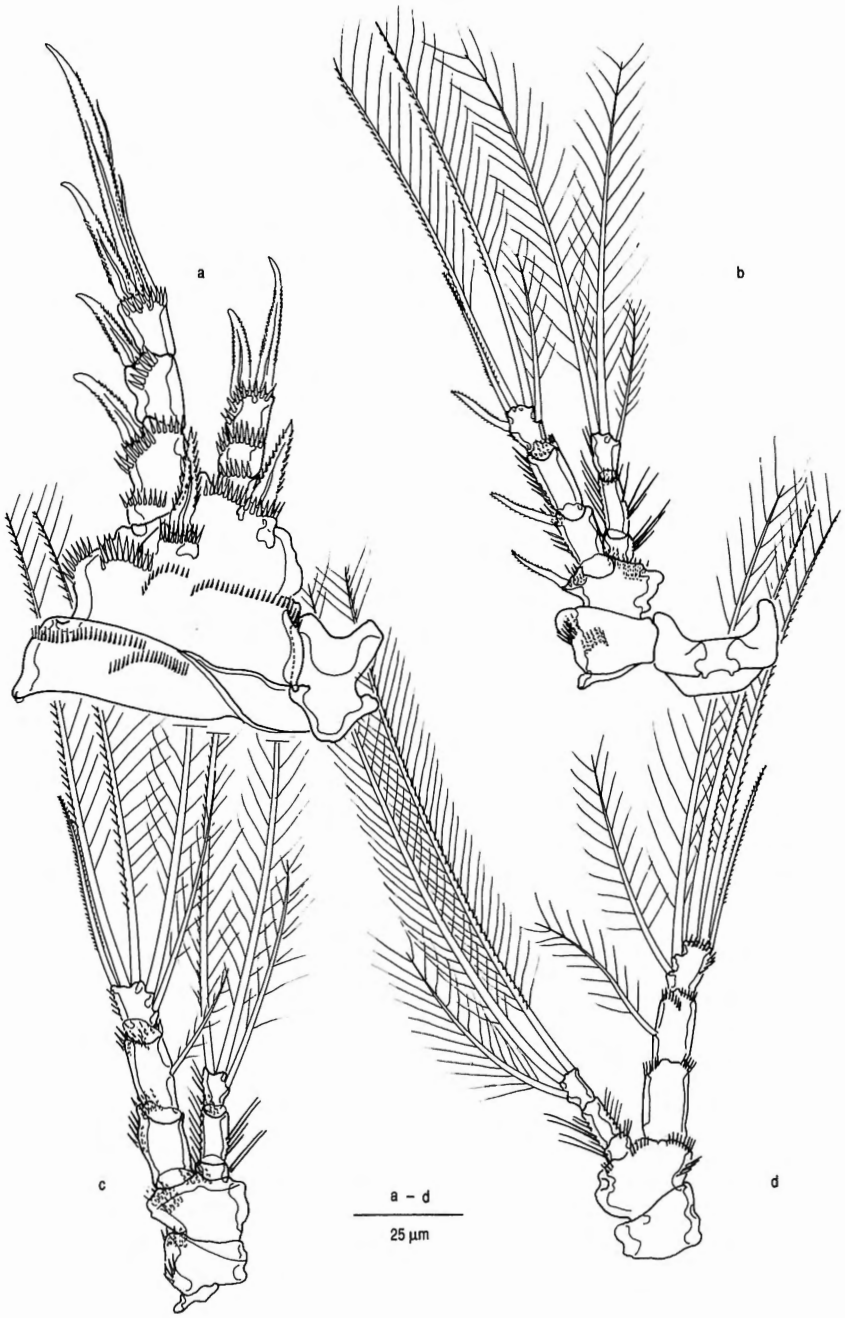

Fig. 3. — *Laubieria tercera* n. sp. : a, P1 ; b, P2 ; c, P3 ; d, P4 (all drawn from the female specimen ; a and d in anterior view, b and c in posterior view).

a slender seta on the dorsal surface ; aesthetasc accompanied by three setae and one thick, smooth spine ; fourth and fifth segments each with a single seta ; ultimate segment bearing a lateral seta, two sub-apical setae, articulating on a basal part, and apically, two fused setae, accompanied by a small aesthetasc.

Antenna (Fig. 2d) with allobasis ; exopodite represented as a strong, armed seta arising from a small cylindrical process (Fig. 2e) ; endopodal segment with five spines and a slender seta along the lateral margin ; distal endopodal margin with three robust, armed spines.

Mandible (Fig. 2f) slender and fragile, having two teeth on the gnathobasis and a one-segmented palp, bearing a single feathered seta.

Maxillule (Fig. 2g) with two blunt, curved spines and a slender seta on the arthrite ; coxa, basis and rami extremely reduced ; maxillulary palp cylindrical, bearing a long feathered seta.

Maxilla (Fig. 2h) with three setae on the syncoxa ; basis produced into a curved blunt process, furnished with three setae : two medio-distally and one proximally.

Maxilliped (Fig. 2k) represented as a small cylindrical appendage, nearly twice as long as wide, bearing three setae : two spinulose and one slender and smooth ; outer margin of the segment furnished with spinules.

P1 (Fig. 3a) with a robust appearance ; all segments strongly sclerotized ; coxae and basis with several rows of fragile spinules ; intercoxal plate with curved distal margin ; outer and inner spine of the basis stout and armed ; exopodite and endopodite three- segmented ; all segments set with spinules ; outer exopodal spines armed medially but smooth apically ; setal formula as in Table I.

TABLE I

Setal formula of Laubieria tercera *n. sp.*

	P1	P2	P3	P4
Exo	0-0-022	0-0-122	0-1-221	0-1-221
End	0-0-020	0-0-120	0-0-120	0-0-120

P2 — P4 (Fig. 3b-d, respectively) : praecoxae small, represented as a slender wedge-shaped structure below the coxae ; coxae and bases with strong margins ; basis of P2 with an outer spine, of P3 and P4 without ; exopodites and endopodites three- segmented ; first exopodal segment of P2 with an outer spine ; first and second exopodal segments in P3 and P4 without an outer spine ; inner margins of the endopodal segments set with some long and slender spinules, without inner setae on the proximal and median segments ; setal formula in as Table I.

Both fifth pair of legs (Fig. 2i) fused together, forming a median plate ; distal margin of the P5-complex deeply incised medially showing a tuft of minute spinules

on two small extensions near the incision ; outer seta smooth, about five times as long as the inner spine.

Male : habitus (Fig. 4c) resembling closely that of the female but with narrower abdomen ; length, 380 µm.

Dorsal integumental structures as in the female ; ventral surface of the abdominal segments with a median pattern of stronger spinules, the posteriormost being the longest.

Antennule (Fig. 2b and c) six-segmented, sub-chirocer ; first and second segments shorter than in the female but with an identical ornamentation and setae ; third segment small, protruded and bearing one aesthetasc and three setae ; fourth segment robust, bearing five setae and an aesthetasc ; upper surface showing a transverse distinct ridge in the distal half, reaching from the anteriorly directed margin towards the posteriorly directed one but terminates at small distance of the latter ; ultimate segments as in the female.

Mouthparts and P1 — P4 as in the female ; P5 (Fig. 2j) resembling that of the female showing however considerably longer inner spines — about 2/3 of the setae — and lacking the median tuft of spinules.

P6 (Fig. 4b) symmetrical, without setae or spines on the outer margins.

Discussion

Within the genus, *Laubieria tercera* n. sp. is most closely related to *L. secunda*. Indeed, both congeners share the highly reduced setal formula without outer spines on the proximal and median exopodal segments of P3 and P4. These features clearly distinguish both species from *L. corallicola* which bears outer spines on the proximal exopodal segments in these legs.

L. tercera n. sp. can easily be distinguished from its closest relative by the chaetotaxy of the second leg which lacks an inner seta on the median exopodal segment and by the relative lengths of the armature elements on the female P5.

At first sight, *L. tercera* n. sp. could be distinguished from *L. secunda* because of the absence of setae on the inner margins of the first and second endopodal segments of P2 — P4 in the former. However, the exact nature of the inner endopodal setae as shown by WELLS (1967) for *L. secunda* needs reconsideration. By their position and fragility they resemble remarkably the slender spinules found on the endopodites of *L. tercera* n. sp.

If these endopodal setae in *L. secunda* turn out to be spinules, the generic designation of *L. secunda* and *L. tercera* n. sp. can seriously be questioned. Indeed, absence of outer exopodal spines, a rather rare phenomenon within harpacticoids, clearly represents an important evolutionary novelty. Considering the three known species of the genus *Laubieria, L. corallicola* differs strongly from the other species by the presence of outer exopodal spines on the proximal segments, the setae on the inner margin of the proximal endopodal podomeres and the large sharp processus on the female P5. It seems not unlikely that *L. corallicola* is a representative

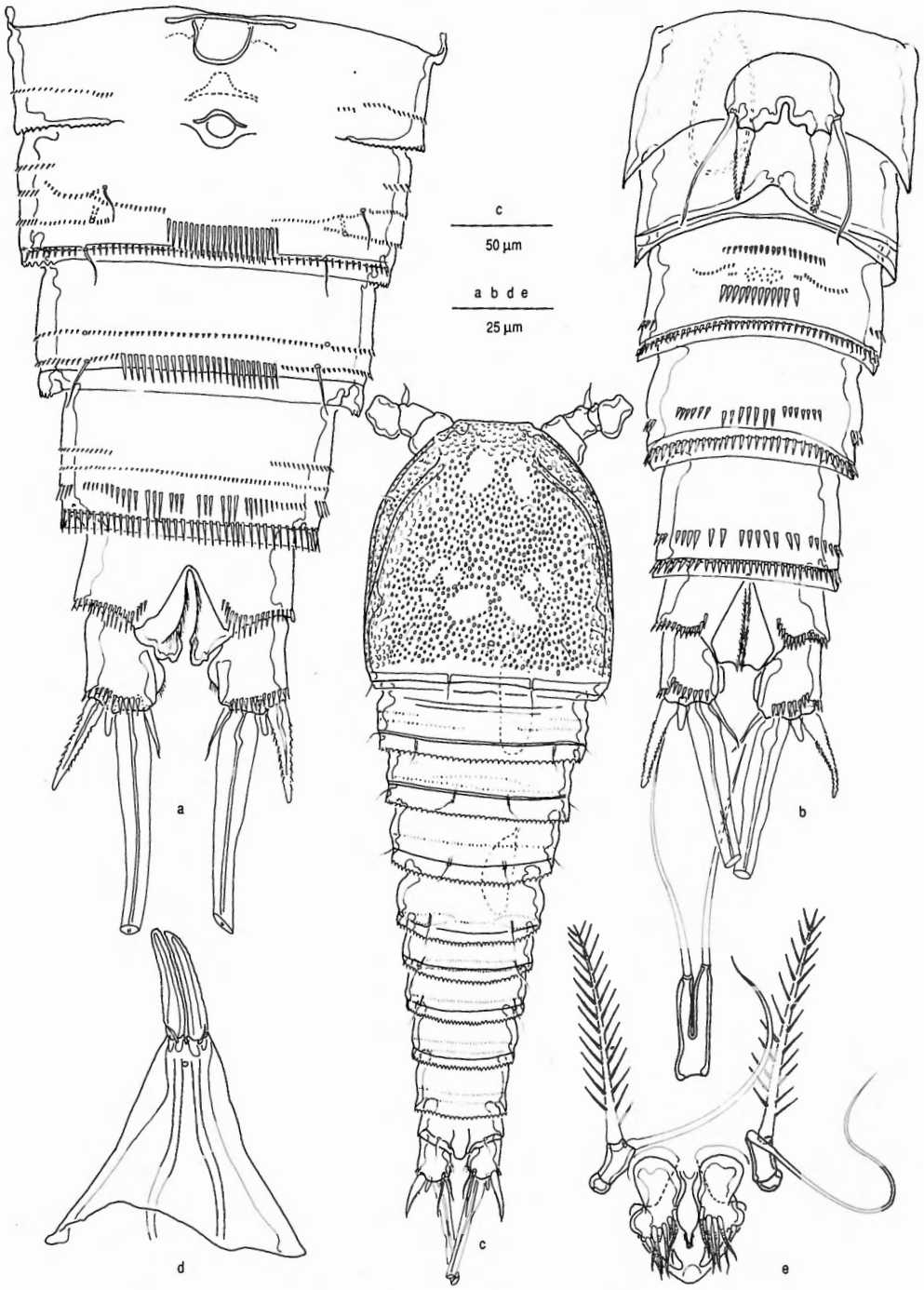

Fig. 4. — *Laubieria tercera* n. sp. : a, female abdomen in ventral view ; b, male abdomen, including P5-bearing somite, in ventral view ; c, male habitus in dorsal view ; *Metis reducta* n. sp. : d, rostrum in ventral view ; e, mouthparts *in situ*.

of a branch phylogenetically situated between the genus *Metis* and the two *Laubieria*-species now known from the Indian Ocean.

genus *Metis* PHILIPPI, 1843

Metis reducta n. sp.

Type material. Holotype, one dissected male, mounted on three slides and labeled COP 3420 A,B and C; paratypes : one dissected female juvenile (copepodid V), mounted on two slides (COP 3419 A and B) and 2 males, 1 female C V and 3 C IV preserved in alcohol (COP 3421).

Type locality. Found in the same sample containing *Laubieria tercera* n. sp.

Etymology. The specific name (Latin : *reductus*) refers to the reduced segmentation of the P1 endopodite.

Description

Male (holotype) : habitus typically Metidae-shaped (Fig. 5a) ; length, measured in lateral view, from the tip of the rostrum towards the distal margin of the furcal rami : 490 µm ; length of cephalothorax about half the body-length ; lateral margins of the body somites tapering towards the anal segment, in dorsal view ; anal operculum rather wide having a distinct convex distal median part.

Integumental structures : surface of cephalothorax and body somites smooth ; thoracic somites without ornamentation along the posterior margins ; posterior margin of abdominal somites set with a row of minute spinules dorsally, and with long strong spinules laterally and ventrally (Fig. 5c) ; hyaline frill of the somites minutely incised.

Rostrum (Fig. 4d) articulating with the cephalothorax and strongly ventrally directed ; lateral margins steep ; rostral tip with two long and strong, curved spine-like structures furnished with lateral strips of hyaline serrated membrane ; two minute sensillae situated ventrally, near the implantation of the curved appendages.

Furcal rami (Fig. 5b) as long as wide ; dorsal seta, articulating on a single basal part, arising near the inner margin ; distal lateral seta spiniform, about 3.5 times as long as the ramus ; proximal lateral seta absent ; outer principal seta slender, 1.5 times as long as the ramus ; inner principal seta typically swollen and as long as the body length ; inner apical seta smooth, slightly longer than the ramus ; surface set with spinules on the distal outer edge and ventrally, along the distal margin (Fig. 5b- c).

Antennule (Fig. 7c and d) seven-segmented resembling closely that of *M. galapagoensis* MIELKE (1989) ; differing only in the ornamentation of the first segment and in the larger shape of the extension on the second segment.

Antenna (Fig. 6c) with a remarkably short allobasis, slightly longer than wide ; surface of coxa and allobasis smooth, except for a few spinules in the upper half

of the anteriorly directed margin ; exopodite represented as a small spiniform seta ; endopodite with six spines (two of them smooth) and several rows of spinules.

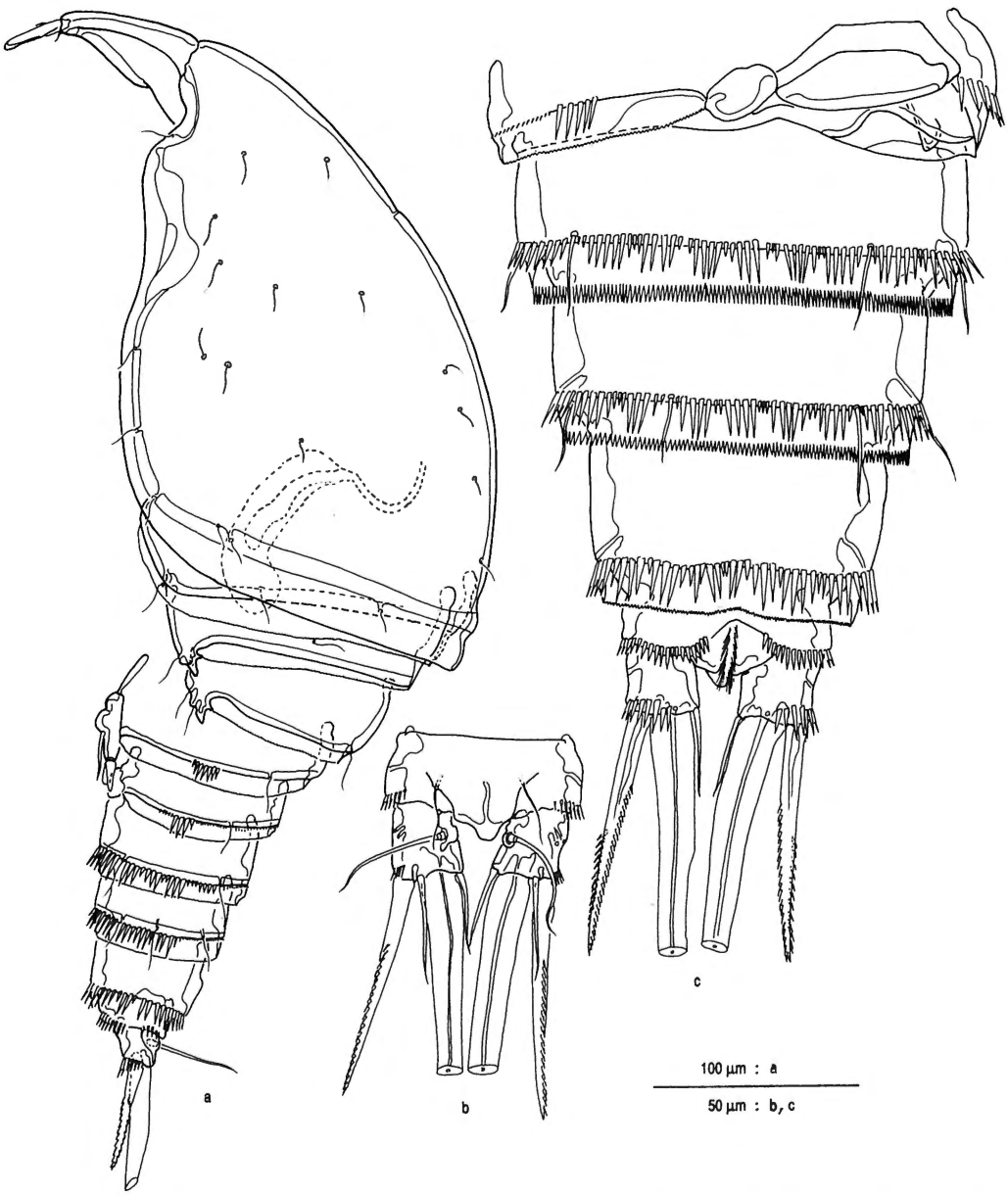

Fig. 5. — *Metis reducta* n. sp. : a, male habitus in lateral view ; b, anal segment in dorsal view ; c, male abdomen in ventral view.

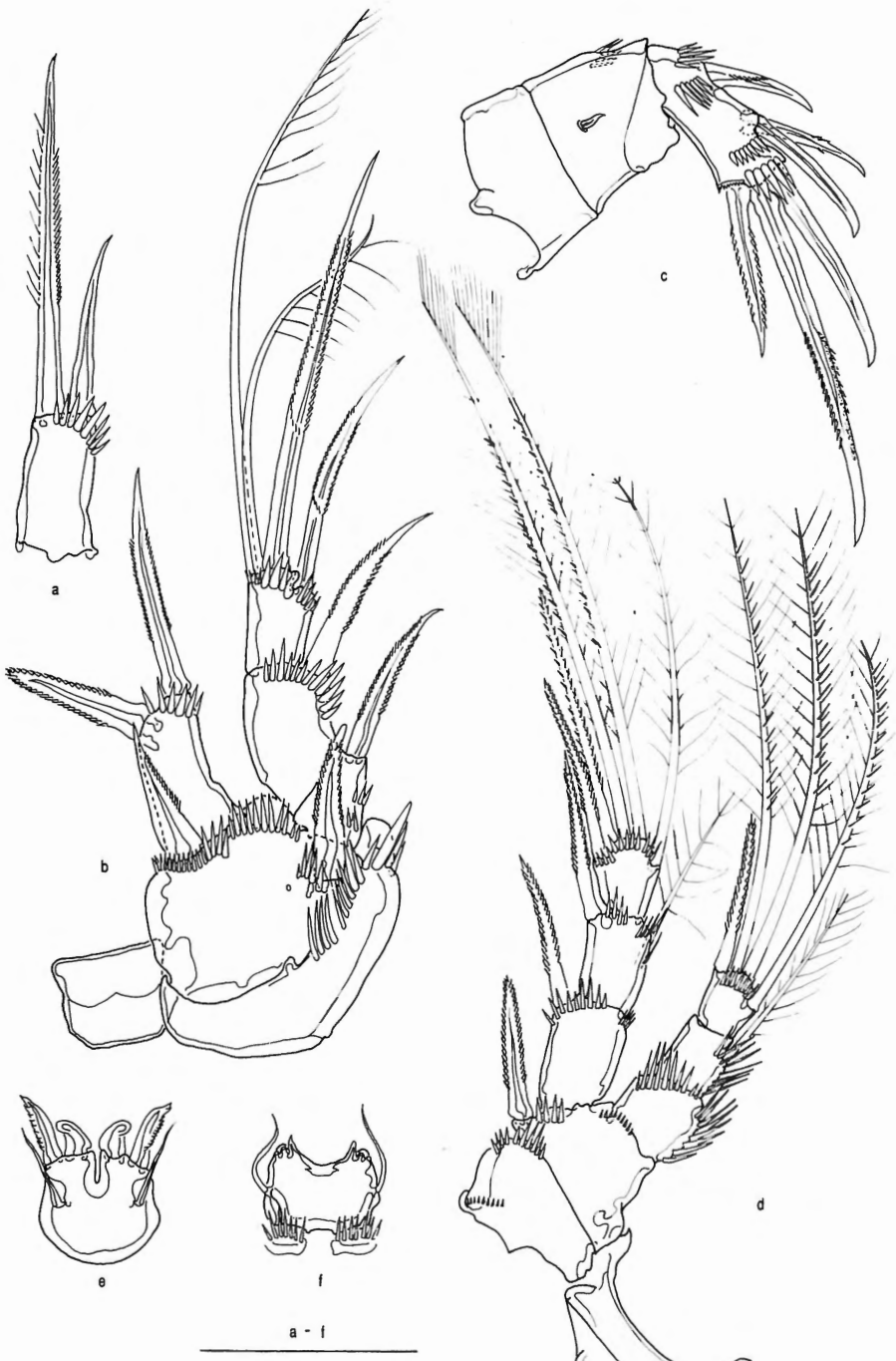

Fig. 6. — *Metis* reducta n. sp. : a, endopodite P1 of female copepodid V ; b, male P1 ; c, antenna ; d, male P2 ; e, male P5 ; f, P5 of female copepodid V.

Mouthparts (Fig. 4e) typically reduced and identical with those of *M. galapagoensis* except for the shorter maxillipedal branches showing no trace of an apical subdivision (Fig. 4e).

P1 (Fig. 6b) strongly sclerotized ; coxa and basis set with rigid spinules ; basis with armed inner and outer spine ; exopodite three-segmented ; proximal segment rather small ; terminal exopodal segment with four appendages ; endopodite one-segmented with, two armed spines, apically ; setal formula as in Table II.

TABLE II

Setal formula of Metis reducta *n. sp.*

	P1	P2	P3	P4
Exo	0-0-022	0-1-122	0-1-222	0-0-222
End	020	0-1-121	1-0-220	1-0-220

P2-P4 (Fig. 6d, 7a and b, respectively) : prae-coxae small, with smooth surfaces ; coxae and bases furnished with a few rows of spinules ; basis of P2 with an outer spine, without in P3 and P4 ; exopodal and endopodal rami three-segmented ; exopodal segments with outer spines ; apical setae on the ultimate endopodal segments plumose and spinulose ; setal formula as in Table II.

P5 (Fig. 6e) represented as a single median plate with a deep incision in the middle of the apical margin, strongly sclerotized ; outer setae distinctly swollen in the proximal half (keel-shaped) ; four apical appendages : outer ones armed, about twice as long as the curved, thick inner ones.

P6 (Fig. 5c) remarkable asymmetrical ; right leg medially situated and nearly circular, left one slightly shorter than half the diameter of the somite, long ovoid and covering a strongly folded region ; armature on neither leg.

Female fifth copepodid : habitus typical ; body with nine somites ; abdominal somites much more tapering than in the male ; fifth leg-bearing somite (145 µm) as wide as the length of the abdomen ; length, measured laterally, 560 µm ; integumental structures of body segments, rostrum and furcal rami as in the male.

Antennule (Fig. 7e) five-segmented with robust first segment and extended second one ; one aesthetasc arising from a distinct socle on the third segment and one aesthetasc implanted on the apical margin of the ultimate segment ; integumental structures of the first and second segments as in the male.

P1 (Fig. 6a) with protopodite and exopodite as in the male ; outer endopodal spine smooth, half as long as the inner one and smooth ; inner spine armed with slender spinules ; general appearance of the endopodal spines obviously more slender than in the male P1.

P2 — P4 as in the male, slightly less sclerotized.

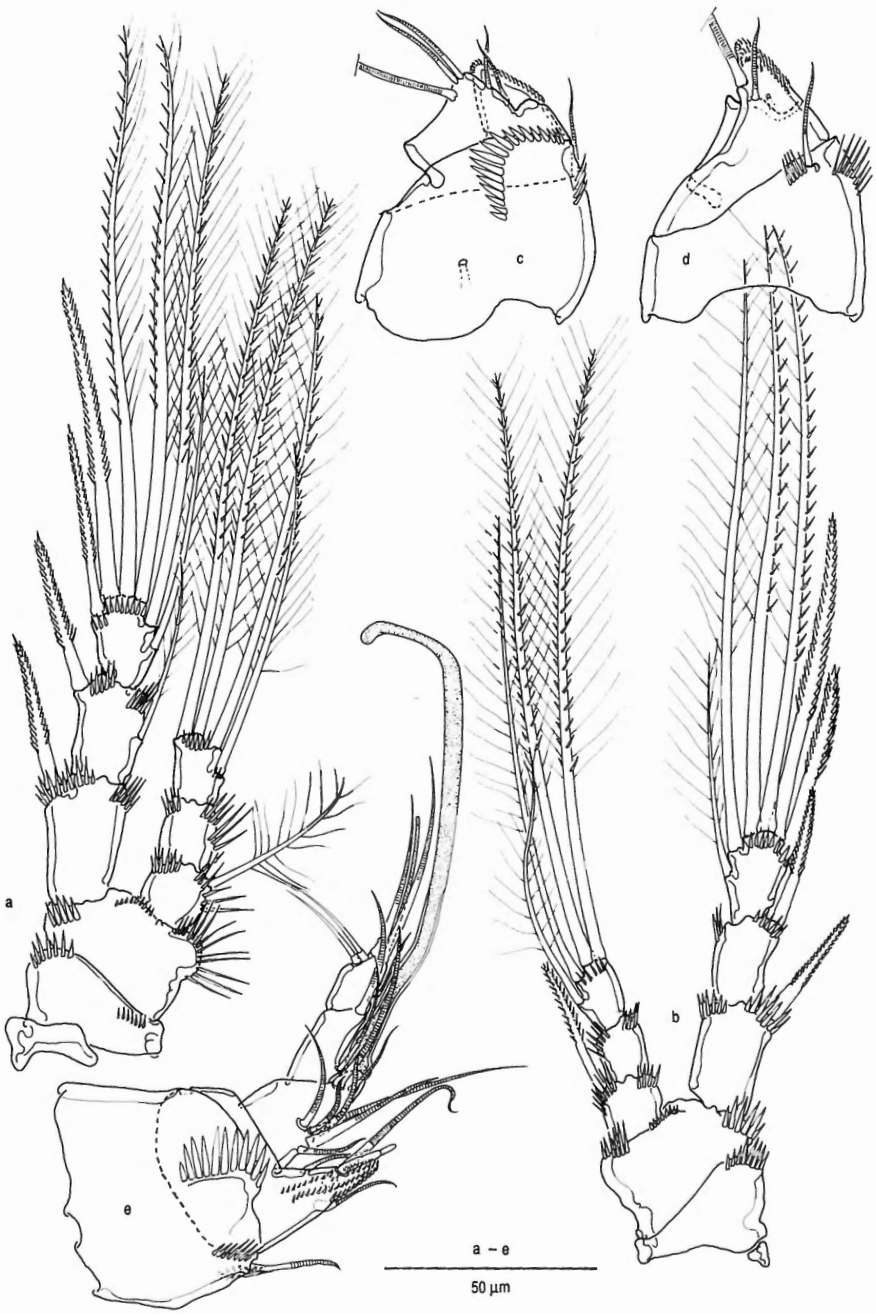

Fig. 7. — *Metis reducta* n. sp. : a, male P3 ; b, male P4 ; c ; first and second segments of male antennule, ventral view ; d, idem, dorsal view ; e, antennule of female copepodid V.

P5 (Fig. 6f) represented as a median plate with a concave anterior margin ; outer setae slender, implanted halfway the lateral margin ; three small apical structures, with a hyaline appearance, on both sides, the inner one sharp, the median and outer ones small and blunt.

Discussion

Although only adult males were encountered in the sample, the difference — i.e. the one-segmented endopodite in P1 — between the *M. reducta* n. sp. and the five other members of the genus is obvious. Notwithstanding the female of *M. reducta* n. sp. is known from the fifth copepodid morphology only, it seems beyond doubt that the adult stage shows this species' specific feature. In general, the fifth copepodid stage possesses legs with the adult ramal segmentation (FERRARI, 1988). Moulting to the adult stage does not affect the general morphology of the leg except for the ornamentation of the setae/spines and podomeres. Therefore, it seems highly possible that the adult female of *M. reducta* n. sp. has a one-segmented P1 endopodite as in the male.

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ÉVOLUTION DU CONTENU ÉNERGÉTIQUE DES OUVRIÈRES ET DES SEXUÉS DE LA FOURMI *LEPTOTHORAX UNIFASCIATUS* (LATREILLE) (*HYMENOPTERA ; FORMICIDAE*) AU COURS DE LEUR DÉVELOPPEMENT

par

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RÉSUMÉ

Les poids secs, contenus énergétiques, pourcentages de lipides et teneurs en cendres ont été mesurés pour les différentes castes de *Leptothorax unifasciatus* jusqu'à l'imago âgé. L'évolution de ces paramètres montre plusieurs différences selon les castes. Les ouvrières accumulent des lipides pendant la vie nymphale, probablement destinés au nourrissage futur des jeunes larves. Le contenu énergétique des mâles diminue continuellement depuis le stade prénymphe et les adultes sont pratiquement vidés de leur contenu lipidique. Les reines accumulent une grande quantité de lipides en fin de vie larvaire mais également après l'émergence. Ces différences sont discutées dans le cadre des modalités reproductives de cette espèce (vol nuptial et fondation indépendante). Des éléments laissent supposer que le coût des sexués et l'effectif des ouvrières déterminent le moment où une société commence à produire les sexués au cours de son développement.

Mots-clés : Contenus énergétiques, développement, castes, *Leptothorax unifasciatus*

Energetic changes during the development of workers and sexuals of the ant *Leptothorax unifasciatus* (LATREILLE) (Hymenoptera ; Formicidae)

SUMMARY

Dry weights, energetic contents, fat and ash percentages were measured for the various castes of *Leptothorax unifasciatus* from egg to old imago. The changes of these parameters indicate many differences according to castes. Workers store fats during the pupal life, which are probably intended for the future feeding of young larvae. The energetic content of males

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decreases continuously from the prepupal stage and adults are virtually emptied of their fat content. Queens store a great amount of fat not only at the end of larval growth but after imaginal emergence as well. These differences are discussed in the context of the reproductive modalities of the species (nuptial flight and queen independent nest founding). Some facts indicate that the energetic cost of sexual making and the number of workers determine the time when a society begins to produce sexuals during its development.

Key-words : Energetic contents, development, castes, *Leptothorax unifasciatus*

INTRODUCTION

Depuis quelques années, on assiste à un regain d'intérêt pour la bioénergétique des insectes sociaux et plus particulièrement des Hyménoptères sociaux. Cette discipline apparaît en effet prometteuse par la manière dont elle peut aborder divers problèmes dont certains sont devenus autant de classiques dans l'étude des insectes sociaux. Ainsi, en analysant comment une société utilise l'énergie qu'elle prélève, la bioénergétique devrait permettre d'étudier sous un nouvel angle la dynamique de croissance et la maturation d'une société, phénomènes pour lesquels il n'existe actuellement que de simples modèles basés sur des données purement numériques (BRIAN, 1965, 1983). Cette perspective est importante parce qu'elle touche à deux questions considérées comme majeures de la biologie des insectes sociaux (PASSERA et KELLER, 1987, 1988), soit, d'une part, à la quantité relative des ressources disponibles que la société doit investir à un moment donné dans la production des sexués et d'autre part, à la quantité relative d'énergie investie dans les sexués mâles et femelles.

Enfin, la bioénergétique permet d'étudier d'autres problèmes comme le type de relation qui existe entre l'investissement en énergie dans les sexués et le mode de fondation d'une société (KELLER et PASSERA, 1988, 1989a ; PASSERA et KELLER, 1988), l'existence ou non d'un vol nuptial (PASSERA *et al.,* 1989), ou encore la production d'une société de Fourmis, dans le cadre d'un bilan énergétique (MARTIN, 1990 ; 1991b). Le présent travail fait partie d'une étude plus générale qui consiste à établir le bilan énergétique de *Leptothorax unifasciatus* (LATREILLE) (MARTIN, 1990), pour lequel la connaissance des contenus énergétiques du couvain et des adultes est indispensable. Sans nous limiter à cet aspect purement quantitatif, nous verrons comment nos données énergétiques permettent d'aborder les différents types de problématiques cités ci-dessus.

MATÉRIEL ET MÉTHODES

24 nids ont été récoltés à Treignes (Namurois, Belgique) en 1986. Après récolte sur le terrain, la reine et les ouvrières sont tuées à l'acétate d'éthyle et les larves sont placées dans des flacons différents correspondant chacun à une classe de taille (intervalle de 0,05 mm). Les échantillons sont déshydratés jusqu'à obtention d'un poids constant (étuve à 70° C, environ 3 h) et conservés dans des flacons en verre scellés à la flamme après y avoir incorporé un morceau de silicagel.

Le contenu calorifique des échantillons est déterminé grâce à la micro-bombe calorimétrique balistique de PHILLIPSON (1964) (PETRUSEWICZ et MACFADYEN, 1970 ; PAINE, 1971 ; PRUS, 1975), après étalonnage avec de l'acide benzoïque de contenu calorifique connu (26,506 J/mg) (précision des mesures de 5 %). Les échantillons sont pressés en pilule de 1 à 20 mg avant la combustion.

En raison de la sensibilité de la méthode (1 mg de poids sec d'échantillon minimum), de la petitesse des sociétés (effectif de 62 à 679 ouvrières et de 30 à 591 larves pour les sociétés récoltées avec reine sur le terrain) et étant donné le faible poids des larves, il a été nécessaire d'opérer des regroupements afin de fournir suffisamment de matériel biologique pour pouvoir faire une mesure du contenu calorifique. Ces regroupements correspondent, pour les larves (1) (Tableau 1 ; L1, Figs 2, 3, 4), aux premier et deuxième stades larvaires (0,50 à 0,75 mm), et pour les larves (2) (Tableau 1 ; L2, Figs 2, 3, 4), au troisième stade larvaire (larves commençant à avoir leur meconium fonçé, 0,95 à 1,20 mm). De même, bien que des mesures individuelles aient été faites pour le poids sec des reines fondatrices, il a fallu opérer un regroupement pour déterminer le contenu calorifique, ce qui n'a permis qu'une mesure globale. A cause de ces divers regroupements et de la quantité limitée du matériel biologique, il n'a pas été possible de faire plus d'une mesure pour chaque catégorie, ce qui nous prive d'informations sur la variabilité des résultats.

Les différentes castes des prénymphes sont bien circonscrites et sont distinguées d'après leur taille (1,70 à 2,40 mm pour les ouvrières, 2,45 à 3,00 mm pour les mâles et 3,05 à 3,45 mm pour les reines). Les castes des larves en fin de croissance ne sont malheureusement pas aussi aisément distingables. Dans le cas des ouvrières, un échantillon de larves de 2,00 à 2,50 mm n'est pas représentatif car il contient non seulement des larves ouvrières en fin de croissance mais aussi des larves mâles et reines en pleine croissance. Le même type de remarque peut être fait pour les larves mâles. Pareil échantillonnage pourrait créer un biais dans la mesure où la composition des larves en fin de croissance peut être différente des larves en phase de croissance. Pour cette raison, seules les prénymphes ont été prises en compte, en supposant leur composition semblable aux larves en fin de croissance (teneur en lipides et contenu énergétique). A l'appui de cette hypothèse, les résultats obtenus par PASSERA et KELLER (1987, 1988) pour *Iridomyrmex humilis* (MAYR) montrent que la composition des larves en fin de croissance est peu différente de celle des prénymphes (teneur en lipides et contenu énergétique par individu légèrement supérieurs à ceux des prénymphes).

Les nymphes d'ouvrières sont séparées d'après leur âge qui se reflète par leur couleur (jeunes nymphes « blanches » à yeux blanc à noir, nymphes âgées « colorées » avec l'apparition de la bande noire abdominale transversale caractéristique de l'espèce indiquant une mue proche). Par manque d'effectif, cette distinction n'a pas été faite pour les autres castes. Les reines sont classées en fonction de leur âge : jeunes reines supposées proche de la mue imaginale (observées le 26/8/86 pour la première fois dans les prélèvements sur le terrain), proche de l'essaimage (observées trois semaines plus tard dans les prélèvements sur le même site naturel), reines âgées (fondatrices de 16 sociétés prélevées avec reine).

En raison de la grande richesse en lipides des femelles ailées, et parfois des prénymphes et nymphes, le pressage de la pilule peut s'accompagner de la perte d'un liquide gras. Celui-ci est recueilli sur un morceau de papier filtre préalablement pesé et de contenu calorifique connu. Après correction adéquate, la combustion en bombe calorimétrique indique que ce liquide correspond effectivement à des lipides en raison de son contenu calorifique particulièrement élevé (40,66 J/mg pour le liquide recueilli des femelles ailées et 39,77 J/mg en moyenne pour les lipides — PETRUSEWICZ et MACFADYEN, 1970 —). Le contenu calorifique des échantillons est corrigé en fonction de la quantité de liquide gras perdu.

Les pilules d'échantillons d'imagos sont particulièrement fragiles en raison de la cuticule très lisse des adultes et à cause de la libération de corps gras lors du pressage. Afin d'y remédier, elles ont été enrobées avec du paraloïd B72 (copolymère de polyméthyl-métacrylate — Plexiglass — et d'éthyl-métacrylate — proportions respectives de 3:7 — dilués à 10 % dans du xylène) qui forme un vernis très solide, facilement inflammable et ne produisant quasi pas de cendres après combustion. Une mesure du contenu calorifique du paraloïd permet de corriger la valeur globale obtenue pour l'échantillon enrobé.

En raison du faible poids larvaire, les échantillons (consistant chacun en une classe de taille) sont pesés globalement avec une micro-balance Sartorius (± 1 µg) ; les poids individuels correspondent donc à une valeur moyenne (effectif des classes de taille toujours supérieurs à 50 individus jusqu'à la limite de classe de 1,75 mm et inférieurs à 25 individus à partir de la limite de classe de 1,85 mm. Dans ce dernier cas, l'effectif moyen est de 8 larves, min. 1, max. 25 larves).

Le pourcentage de lipides par rapport au poids sec est estimé à partir du contenu énergétique exprimé par mg *ash free,* en supposant qu'un milligramme de lipides est équivalent à 39,35 J et que le résidu sec (*lean dry weight*), composé d'hydrates de carbone et de protéines, vaut, en moyenne, 18,84 J/mg (PEAKIN, 1972, MOROWITZ, 1968). En cela, nous faisons la démarche inverse de PEAKIN (1972) et de PASSERA et KELLER (1987, 1988, entre autres) qui estiment le contenu calorifique de leurs échantillons en mesurant leur teneur en lipides.

RESULTATS

Poids sec

L'évolution du poids sec des larves relativement à leur longueur montre une régularité remarquable jusqu'à la taille 1,85 mm (Fig. 1). A un tel phénomène correspond une relation simple liant le poids (P) à la longueur (L) telle $P = a.L^x$ où x est le facteur indiquant comment L est liée à P, et a est le coefficient de proportionnalité, soit :

$$P = 43{,}85.L^{3{,}061} \quad (r = 0{,}994)$$

Avec x valant 3,061, cette relation indique une croissance larvaire isométrique. Au delà de 1,85 mm, l'évolution devient plus irrégulière et, étant donné ce phénomène,

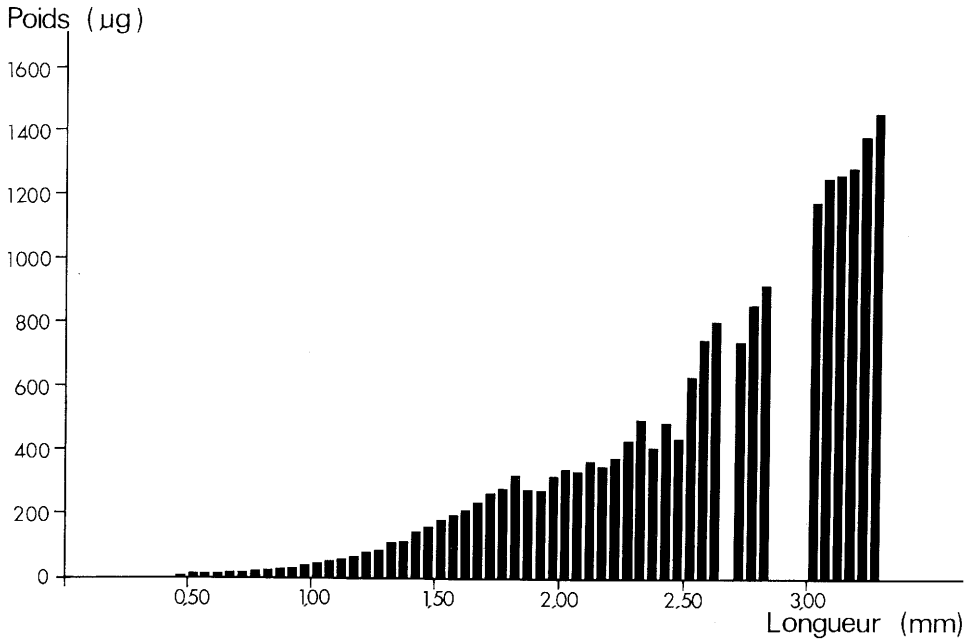

Fig. 1. — Relation liant le poids sec à la longueur des larves « actives » (consommatrices de nourriture ; 3844 larves, 53 mesures).

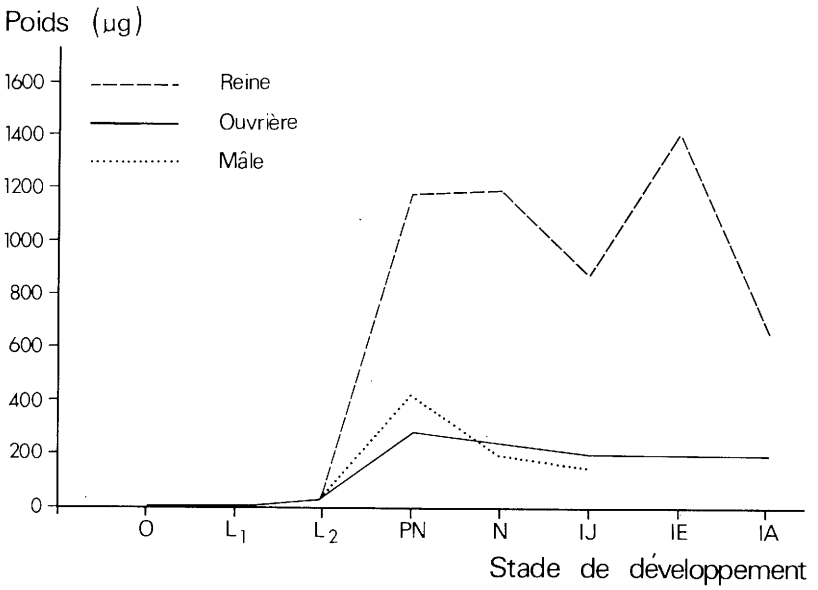

Fig. 2. — Evolution du poids sec des reines, mâles et ouvrières. Les abscisses se réfèrent au tableau 1 (O : oeufs ; L1 : larves (1) ; L2 : larves (2) ; PN : prénymphe ; N : nymphe ; IJ : imago juvénile ; IE : imago au moment de l'essaimage ; IA : imago âgé.

TABLEAU 1

Evolution du poids sec, du contenu énergétique, du pourcentage de lipides (par rapport au poids sec) et des cendres des ouvrières, mâles et reines de Leptothorax unifasciatus.

		Œufs	Larves		Pré-nymphes	Nymphes		Imagos		
			(1)	(2)		Jeunes	âgées	jeunes	essaim.	âgés
Ouvrières	µg	4,9	6,3	28,6	293,9	260,6	239,7	209,4		214,0
	J/ind.	0,13	0,2	0,8	8,2	7,7	7,6	6,1		5,7
	J/mg ps	25,9	24,6	28,4	26,3	29,4	31,7	29,2		26,5
	J/mg ps (*ash free*)	27.1	25,4	29,2	26,8	30,0	32,3	29,8		27,0
	% lipides	40	32	50	39	54	66	53		40
	% cendres	4,3	3,3	2,5	2,1	1,9	2,0	1,9		2,0
	N	2149	936	968	70	50	42	45		1131
Males	µg				432,8	207,0		159,2		
	J/ind.				11,2	4,6		3,4		
	J/mg ps				25,5	22,4		21,5		
	J/mg ps (*ash free*)				26,0	23,0		22,1		
	% lipides				35	20		16		
	% cendres				2,0	2,8		2,5		
	N				16	37		26		
Reines	µm				1184	1207		883,1	1414,2	657,2
	J/ind.				36,4	34,9		25,1	40,2	17,6
	J/mg ps				30,7	28,9		28,5	31,0	26,8
	J/mg ps (*ash free*)				31,2	29,3		28,9	32,1	27,5
	% lipides				60	51		49	65	42
	% cendres				1,9	1,7		1,8	1,8	1,2
	N				24	11		9	14	16

(1) : petites larves (1er et 2^{e} stades larvaires) ; (2) : larves de taille moyenne (3^{e} stade avec le meconium qui commence à ce colorer) ; « essaim. » : reines adultes au moment de l'essaimage ; N : nombre total d'individus dans l'échantillon.

la même relation établie pour l'ensemble des larves s'en ressent, de sorte que l'isométrie est moins bien vérifiée :

$$P = 43{,}02.L^{2{,}839} \quad (r = 0{,}994)$$

D'une manière générale, le poids sec des ouvrières, reines et mâles décroît depuis le stade prénymphal jusqu'à l'émergence, avec, dans le cas des mâles, une chute importante entre les stades prénymphe et nymphe (Tableau 1, Fig. 2). Après l'émergence, le poids des ouvrières varie peu puisque les ouvrières âgées sont à peine plus lourdes que les plus jeunes (Tableau 1). Par contre, le poids des jeunes reines augmente fortement après l'émergence pour devenir jusqu'à 1,6 fois plus élevé que sa valeur initiale.

Pourcentage de lipides par rapport au poids sec

Si le début de la croissance larvaire (L1) se traduit par une légère diminution du pourcentage de lipides par rapport au stade oeuf, les lipides redeviennent plus abondants au cours de la vie larvaire « active » (larves consommatrices de nourriture ; voir L2 — Fig. 3, Tableau 1 —). Lorsque les larves ont atteint une taille moyenne (L2 — Fig. 3), leur teneur en lipides a tendance à diminuer tant pour les larves ouvrières que pour les larves mâles. Ce n'est cependant pas le cas chez les

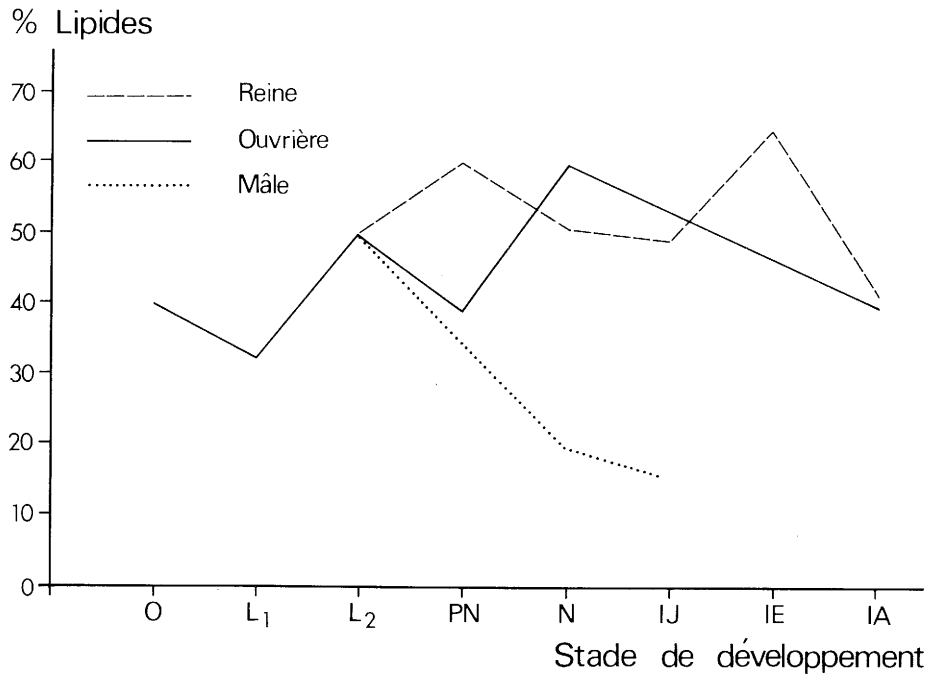

Fig. 3. — Evolution du pourcentage de lipides par rapport au poids sec des reines, mâles et ouvrières. Légendes : voir Fig. 2.

larves reines qui continuent à augmenter leur contenu lipidique. Il en résulte que le stade prénymphe des reines est le plus riche en lipides, excepté le stade des reines adultes proches de l'essaimage qui accumulent une quantité spectaculaire de lipides après l'émergence. Chez les mâles, le pourcentage de lipides diminue brutalement dès le stade prénymphe et ce phénomène se poursuit jusqu'à l'émergence. Par contre chez les ouvrières, le stade nymphal se caractérise par un accroissement du pourcentage de lipides.

Contenu énergétique par individu

Son évolution est très semblable à celle du poids sec (Fig. 4). Le point important consiste en la perte constante de contenu énergétique depuis le stade prénymphal jusqu'au stade adulte âgé ; cependant, les jeunes reines augmentent considérablement leur contenu énergétique après l'émergence jusqu'à l'essaimage, acquérant en moyenne 531 µg de poids sec. Si on suppose que cette prise de poids est essentiellement due à une accumulation de lipides, on peut calculer (compte tenu du contenu calorifique lipidique moyen de 39,35 J/mg) qu'elle correspond à 20,9 J. La relativement bonne concordance avec les 15,1 J réellement observés suggère qu'il en est bien ainsi.

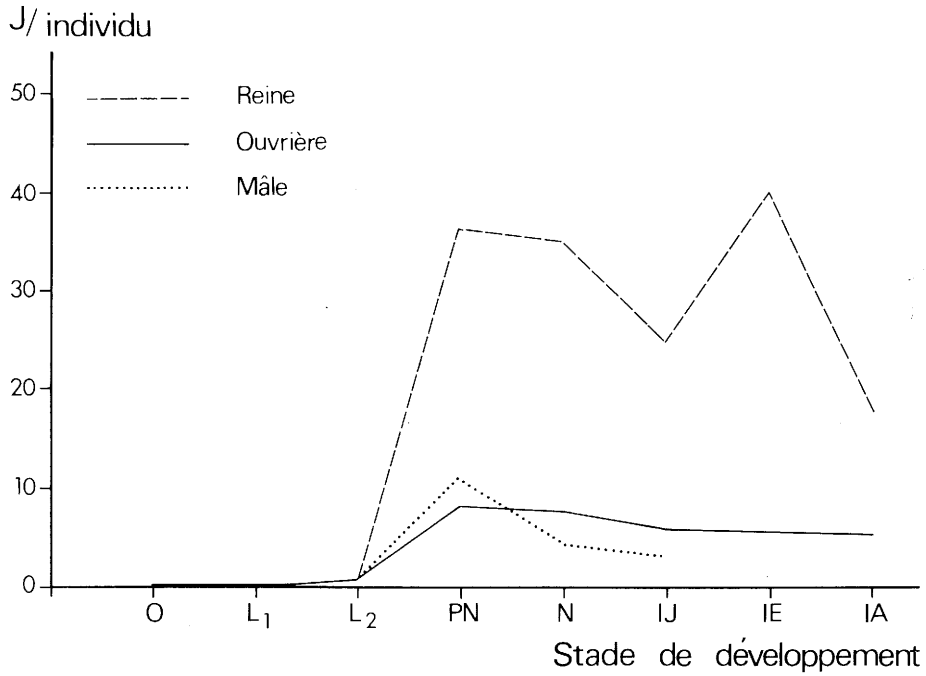

Fig. 4. — Evolution du contenu énergétique des reines, mâles et ouvrières. Légendes : voir Fig. 2.

Teneur en cendres

En vue de comparaisons interspécifiques, il est courant d'exprimer les contenus calorifiques « sans cendres » (*ash free*) (PAINE, 1971). La teneur en cendres est faible pour l'ensemble des catégories étudiées, inférieure à 4 %, en général (Tableau 1).

DISCUSSION

Poids sec larvaire

La croissance larvaire répond relativement bien à une relation isométrique jusqu'à une taille de 1,85 mm au-delà de laquelle elle devient plus irrégulière. Trois éléments peuvent expliquer cette irrégularité.

Premièrement, la petitesse des échantillons au-delà de 1,85 mm peut créer une plus grande variabilité. En effet, comme précisé dans « Matériel et méthodes », l'effectif des classes de taille est toujours supérieur à 50 individus jusqu'à la limite de classe de 1,75 mm, pour être ensuite inférieur à 25 à partir de la limite de classe 1,85 mm.

Deuxièmement, il est intéressant de remarquer que cette irrégularité coïncide précisément avec l'apparition dans ces classes de taille des premières larves entamant la prénymphose. Il est raisonnable de supposer que l'arrêt de la prise de nourriture ainsi que les modifications physiologiques précédant la prénymphose provoque un amaigrissement des larves, ce qui doit se répercuter dans la relation poids — longueur. Etant donné qu'à partir de ces tailles larvaires il y a constamment un chevauchement entre les larves qui continuent une croissance normale (larves à devenir mâle et femelle royale) et celles qui commencent à se préparer à la prénymphose, la régularité observée en deça de la limite de classe 1,85 mm dans la relation poids — longueur n'est plus observable au delà.

Enfin troisièmement, ainsi que le mentionne PLATEAUX (1970) pour *Leptothorax nylanderi* (FÖRSTER), une croissance isométrique n'est plus vérifiée chez les larves mâles en fin de croissance, qui deviennent plus longues et cylindriques que leurs équivalents ouvrière et reine. Cette hétérogénéité larvaire observable également chez *L. unifasciatus* contribue probablement à affaiblir la relation isométrique au delà des tailles 1,85 mm.

Evolution du contenu énergétique des ouvrières

Le point le plus significatif dans l'évolution du contenu énergétique des ouvrières consiste en l'accumulation de corps gras au cours de la vie nymphale de sorte que leur teneur en lipides atteint sa valeur maximale à ce moment. Il en résulte que les jeunes ouvrières sont plus riches en énergie que les ouvrières plus âgées. Pareilles observations ont été faites par MACKAY (1985) chez *Pogonomyrmex subnitidus* EMERY et *P. rugosus* EMERY, et par PASSERA et KELLER (1987, 1988) chez *Iridomyrmex humilis*.

Il est très probable que le surplus d'énergie dont disposent les jeunes ouvrières soit rendu à la société sous forme de nourriture spécifique aux jeunes larves. En effet, ainsi qu'en témoigne la couleur opalescente de leur meconium, les larves des premiers stades ne reçoivent pas de fragments de nourriture carnée comme les larves plus âgées. Elles sont nourries d'oeufs alimentaires pondus par les ouvrières et probablement du contenu des glandes pharyngiennes de ces dernières (MARTIN, 1990). L'observation n'a pas permis de préciser si la ponte d'oeufs alimentaires résultait essentiellement des jeunes ouvrières. On sait cependant que, chez *Myrmica* LATREILLE (BRIAN, 1983), le développement des glandes ovariennes à vitellus et des glandes pharyngiennes est précisément une caractéristique des jeunes ouvrières qui restent au nid et exercent des tâches de nourrices. De plus, il est bien connu que les jeunes ouvrières qui ne fourragent pas encore ont des ovaires actifs et sont, en général, bonnes pondeuses (ABBOTT, 1978 ; PASSERA et KELLER, 1987, 1988), bien que dès leur naissance, certaines ouvrières soient prédisposées à une plus grande fécondité que d'autres (PLATEAUX, 1970). Chez *Formica polyctena* FÖRSTER (HORSTMANN, 1982), les ouvrières qui restent au nid ont les ovaires et les corps gras beaucoup plus développés que les fourrageuses, et chez *Pogonomyrmex subnitidus* et *P. rugosus* (MACKAY, 1985), les jeunes ouvrières (*callows*) ont une teneur en lipides plus élevée que les ouvrières plus âgées.

Il semble donc que le surplus d'énergie stocké par les jeunes ouvrières au cours de leur développement larvaire leur permette, dès l'émergence, d'atteindre une efficacité de nourrice optimale par la production immédiate de nourriture spécifique aux jeunes larves. Cette caractéristique est encore accentuée par la remarquable coïncidence qui existe, chez *L. unifasciatus* (MARTIN, 1988, 1990), entre l'émergence des jeunes ouvrières et l'éclosion massive de jeunes larves, issues de la ponte royale printanière.

Il doit être clair, cependant, qu'il n'y a pas, à proprement parler, d'accumulation de corps gras au stade nymphal dans la mesure où les nymphes ne se nourrissent plus. Il est probable que celles-ci ont accumulé, à la fin de leur vie larvaire, des réserves énergétiques qui sont transformées pendant la nymphose sous forme de lipides pouvant servir ultérieurement de nourriture pour les jeunes larves. Ceci apparaît donc comme un véritable investissement dans une génération pour la génération suivante. D'un point de vue énergétique, cet investissement pourrait sembler, à première vue, comme un gaspillage en raison d'un niveau supplémentaire dans les transformations énergétiques (nourriture, larve, larve) et de pertes à chaque étape du processus. Pourtant, ce type de transfert existe également chez les Termites (JOSENS, comm. pers.) et il est probable que le coût élevé de la production de nourriture spécifique aux larves soit largement compensé par l'abaissement de la mortalité larvaire (en raison d'une nourriture parfaitement assimilable, sans risque de contaminations). Le gain énergétique obtenu par la diminution de la mortalité deviendrait alors plus grand que la perte dans le transfert d'énergie d'une génération à la suivante.

Evolution du contenu énergétique des mâles

Contrairement aux ouvrières et aux sexués femelles, les mâles sont caractérisés par une chute continue de leur contenu énergétique dès le stade prénymphe. Ce phénomène a également été observé chez diverses espèces, telles *Iridomyrmex humilis* (PASSERA et KELLER, 1987, 1988), *Pogonomyrmex* spp. (MACKAY, 1985), *Lasius niger* L. (BOOMSMA et ISAAKS, 1985) et *Tetramorium caespitum* (L.) (PEAKIN, 1972).

La diminution considérable du contenu énergétique entre les stades prénymphe et nymphe peut être interprétée comme le résultat de l'investissement énergétique dans l'édification de l'appareil reproducteur et des structures permettant l'essaimage, tels les muscles alaires. Or, cette diminution s'accompagne de taux respiratoires très élevés chez les nymphes de mâles (MARTIN, 1990 ; 1991a), supérieurs aux taux observés chez les nymphes d'ouvrières, et qui témoignent d'un important métabolisme. La forte diminution du contenu énergétique des mâles semble logique dans la mesure où il ne serait pas avantageux pour la société d'investir de l'énergie dans les mâles qui sont destinés à une mort rapide après l'essaimage et la fécondation des jeunes reines.

Il apparaît, de plus, que les mâles proches de l'essaimage sont pratiquement « vidés » de leur contenu lipidique. Il est probable que les lipides soient convertis en « carburant » spécifique au vol nuptial car il est maintenant connu que les mâles des espèces pratiquant un essaimage reproductif accumulent de grandes quantités d'hydrates de carbone, principalement sous forme de glycogène (PASSERA *et al.*, 1989).

Evolution du contenu énergétique des reines

Par comparaison avec les ouvrières, les reines présentent trois différences majeures : premièrement, elles continuent à s'enrichir en lipides pendant la fin de la vie larvaire, de sorte que le contenu énergétique atteint au stade prénymphal un maximum qui n'est dépassé que par le stade jeune reine essaimante ; deuxièmement, les reines n'accumulent pas de corps gras au cours de la vie nymphale ; et troisièmement, les jeunes reines adultes emmagasinent une importante quantité de lipides depuis l'émergence jusqu'à l'essaimage.

En ce qui concerne l'absence d'accumulation de lipides au cours de la vie nymphale, PASSERA et KELLER (1988) ont observé ce phénomène chez *I. humilis* et ils estiment que l'édification du système reproducteur en est la cause.

Les deux autres différences peuvent s'expliquer en fonction des modalités de la reproduction et de la fondation d'une nouvelle société. En effet, KELLER et PASSERA (1989a, 1989b) ont montré que la teneur en lipides des reines dépendait étroitement du mode de fondation des sociétés. Dans le cas d'espèces à fondation dépendante, le pourcentage de lipides par rapport au poids sec augmente très peu entre l'émergence et le moment de l'essaimage (pourcentages moyens respectifs de 10 et 14 %), ce qui se traduit par une augmentation de 30 % du contenu énergétique initial (à l'émergence). Dans le cas d'espèces à fondation indépendante, la teneur en lipides augmente considérablement depuis l'émergence (16 et 57 %, respectivement), de

sorte que les reines au moment de l'essaimage ont augmenté leur contenu énergétique initial de 284 % ! Enfin, chez *Manica rubida* (LATREILLE), espèce à fondation indépendante non cloîtrée (la reine quitte le nid pendant la fondation pour fourrager), les teneurs en lipides sont intermédiaires et valent respectivement 10 % et 43 % (à l'émergence et à l'essaimage ; 46 % à l'essaimage chez *L. nylanderi*) pour les teneurs en lipides, et le contenu énergétique au moment de l'essaimage a augmenté de 129 % par rapport au contenu initial.

Comme *L. nylanderi, L. unifasciatus* est une espèce à fondation indépendante non cloîtrée. Cette absence de claustration est en fait fort relative car la reine fondatrice de *L. unifasciatus* ne sort que très rarement pour fourrager en cours de fondation (MARTIN, données non publiées). Il devrait en résulter que l'enrichissement en lipides des jeunes reines fondatrices est proche de ce qui est connu pour les espèces à fondation indépendante cloîtrée. Effectivement, les jeunes reines proches de l'essaimage ont une teneur élevée en lipides, soit 65 % (également 65 % chez *Lasius flavus* (FABRICIUS) : WRIGHT, 1989), mais l'accumulation d'énergie entre l'émergence et l'essaimage ne correspond qu'à 60 % du contenu énergétique initial !

Ce paradoxe s'explique par le type particulier de stratégie adopté par *L. unifasciatus*. En effet, contrairement aux cas connus actuellement des Dolichoderinae, Formicinae et Myrmicinae étudiées par KELLER et PASSERA (1989a, 1989b), les reines accumulent une grande quantité de lipides pendant la vie larvaire, de sorte que le taux de lipides est le plus élevé pendant le stade prénymphal (60 % contre 23 % chez *I. humilis* : PASSERA et KELLER, 1987, 1988) et que les adultes ont 49 % de lipides à l'émergence (contre 10 à 16 % dans les autres cas, 12 % chez *I. humilis* : PASSERA et KELLER, 1987) ! Il en résulte que l'accumulation d'énergie entre l'émergence et l'essaimage est beaucoup moins marquée et plus proche des espèces à fondation dépendante comme *I. humilis* (79 % : PASSERA et KELLER, 1987).

Compte tenu des coûts de production d'une ouvrière (12,7 J pour les larves qui n'hibernent qu'une fois : MARTIN, 1990, 1991b), la différence de 22,6 J entre une jeune reine proche de l'essaimage et une reine fondatrice mature peut rendre compte de l'énergie nécessaire à la fondation d'une nouvelle société. Pareille accumulation d'énergie depuis l'émergence jusqu'à l'essaimage est connue également chez les sexués de Termites (BARONI-URBANI *et al.*, 1978) dont les imagos ailés possèdent un contenu énergétique jusqu'à 65 % supérieur à celui des jeunes sexués fondateurs (JOSENS, 1982). Quant aux reines fondatrices matures, elles retrouvent un pourcentage de lipides et un contenu énergétique spécifique semblable à ceux des ouvrières plus âgées.

Coût relatif des sexués

Se basant sur la « *kinship theory* » (HAMILTON, 1964) et la « *sex ratio theory* » (FISHER, 1958), TRIVERS et HARE (1976) suggèrent que, chez les insectes eusociaux, la reine est en conflit avec ses filles ouvrières dans la quantité d'investissement en descendance reproductive (voir PASSERA et KELLER, 1987 ; PASSERA, 1984, et DAWKINS, 1976, pour un exposé de la théorie). En première approximation, ces auteurs ont supposé que le poids sec des sexués était une bonne estimation de cette quantité

d'investissement. PASSERA et KELLER (1987) ont très justement fait remarquer que cette hypothèse était trop simplificatrice et qu'une estimation correcte devait tenir compte non seulement du contenu énergétique des sexués au lieu de leur poids sec mais aussi de l'énergie dissipée dans leur respiration et de l'accumulation lipidique des jeunes reines avant l'essaimage.

Il semble cependant qu'on ait souvent perdu de vue que la kinship theory se base sur des *quantités d'investissement,* ce qui implique non seulement une dimension énergétique mais également une dimension temporelle dans leur évaluation !

En ce qui concerne la dimension énergétique, il est nécessaire d'établir le coût réel que doit supporter une société pour produire les sexués. Ceci suppose la quantification de, non seulement, l'énergie totale utilisée par un individu, mais aussi des éventuels recyclages internes dans la société (consommation des cadavres et d'oeufs alimentaires, par exemple).

Dans le cas de la dimension temporelle, il faut tenir compte du temps de développement éventuellement plus long des reines (ce qui est le cas des *Leptothorax* : PLATEAUX, 1970) et du temps de maturation des jeunes reines qui accumulent des lipides. Ces deux facteurs peuvent être gourmands en temps (quelques jours dans le premier cas mais trois semaines dans le second pour *L. unifasciatus* : MARTIN, données non publiées), et comme ils mobilisent la force ouvrière au profit d'un seul sexe, ils doivent être compatibilisés au même titre que le coût énergétique de production des sexués. Enfin, le sex ratio doit être idéalement établi pour la durée de vie totale d'une société et non pas à un moment donné de son développement. En effet, d'une part les sociétés en phase de croissance commencent à produire les mâles bien avant les reines (jusqu'à trois ans chez *L. nylanderi* : PLATEAUX, 1980, 1982), et d'autre part, la production relative des deux sexes peut fluctuer d'année en année (cas de *L. nylanderi* : PLATEAUX, 1982).

En ce qui concerne *L. unifasciatus,* ce travail ne fournit que les contenus énergétiques. Une comparaison de ceux-ci reste toutefois intéressante mais il faut garder en mémoire que, dans le cas des sexués et en raison de ce qui vient d'être discuté, ils ne consistent qu'en une approximation grossière de la quantité d'investissement réalisée par la société. Pour les raisons exposées dans « Matériel et méthodes », la comparaison est faite entre les prénymphes des différentes castes, moment où le contenu énergétique est supposé le plus proche du maximum.

Par rapport à une ouvrière, un mâle et une reine ont un contenu énergétique maximal respectivement 1,4 fois et 6,2 fois plus élevés, (compte tenu, chez la reine, de l'accumulation de lipides après l'émergence, ce qui porte son contenu énergétique total à 51,5 J). Chez *I. humilis,* ces rapports respectifs sont estimés à 3,1 pour les mâles et 7,5 pour les reines (PASSERA et KELLER, 1987). La faible différence entre le contenu énergétique des ouvrières et des mâles explique vraisemblablement pourquoi une société de *L. unifasciatus* est déjà capable de produire des mâles pendant sa phase de croissance, à partir d'un effectif d'environ cent ouvrières (MARTIN, 1990). Par contre, la production de reines n'apparaît pas avant que la société ait atteint un seuil minimal d'environ 500 ouvrières (MARTIN, 1990), soit 5 fois plus. Or, par rapport à un mâle, une reine a un contenu énergétique maximal 4,6 fois

plus élevé. Ces valeurs similaires sont étonnantes mais, dans l'état actuel des connaissances, il n'est pas possible de préciser si cette coïncidence témoigne d'un phénomène signifiant ou non.

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ALKANOLS IN THE MANDIBULAR GLAND SECRETION OF THE ANT *TETRAMORIUM CAESPITUM*

by

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SUMMARY

The madibular glands of workers of *T. caespitum* contain 4-methyl-3-hexanone and 4-methyl-3-hexanol as major substances, and nonanal, 2-pentanone, 4-methyl-3-heptanol, 4-methyl-3-octanol and 4,6-dimethyl-3-octanol as minor substances. The last three have been newly identified by mass spectrometry and their identification confirmed by synthesis. The probable biosynthetic origins of all these compounds are discussed.

Key words : Hymenoptera, Formicidae, *Tetramorium caespitum*, mandibular glands, alcohols, 4-methyl-3-hexanol.

INTRODUCTION

Many, but by no means all, species of Formicidae contain volatile chemicals in their mandibular glands. The release of these volatile chemicals has often been shown to induce alarm, aggression or attraction in nestmates of the releasers. Although some of these substances, particularly ketones and alcohols (ATTYGALLE and MORGAN, 1984a) may be found in several species in more than one subfamily, they tend to form a specific blend for each species. The correct identification of this pheromonally active secretion can at times be a useful tool in recognizing a species.

MASCHWITZ (1964) observed that crushed heads and mandibular glands of *Tetramorium caespitum* (L., 1758) released « alarm » behaviour in conspecifics. LONGHURST *et al.* (1980) examined the mandibular glands of six species of *Tetramorium* and in three of them, including *T. caespitum*, found 3-octanone as the major substance. In *T. caespitum* it was accompanied by five minor components

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and one major one, all unidentified. PASTEELS *et al.* (1980) reported that heads of *T. caespitum* contained 4-methyl-3-hexanol and that heads of sexuals contained 4-methyl-3-hexanone additionally.

T. caespitum is almost indistinguishable morphologically from *T. impurum* (FOERSTER, 1850) though they are pheromonally distinguishable (ATTYGALLE and MORGAN, 1984b; BILLEN *et al.*, 1986; MORGAN and OLLETT, 1987). PASTEELS *et al.* (1981) corrected their first report when they found they had examined *T. impurum* and not *T. caespitum*. They went on to show that *T. caespitum* also contained 4-methyl-3-hexanol and 4-methyl-3-hexanone (ROISIN, unpublished data ; CAMMAERTS *et al.*, 1985). To see if the difference between the observations of LONGHURST *et al.* (1980) and CAMMAERTS *et al.* (1985) was due to possible racial differences, we examined workers from seventeen colonies of *T. caespitum* from various sites in Western Europe and found in all cases that 4-methyl-3-hexanol was usually the major substance, with small amounts of 4-methyl-3-hexanone, 2-pentanone, (as claimed there) decanal and three unidentified compounds (ALI *et al.*, 1987). In some individuals (from the same nests) there was a large amount of 4-methyl-3-hexanone, but there was no evidence of different races within the species. At the time, equipment to identify the other minor components was not available.

The mandibular glands of *T. caespitum* have now been re-examined by linked gas chromatography-mass spectrometry (GC-MS) and the minor components identified by comparison with synthetic compounds.

MATERIALS AND METHODS

Ants for the present work were collected in Dorset (from where the ants used by LONGHURST *et al.* (1980) were collected) and maintained in artificial colonies in the laboratory as described by ALI *et al.* (1987).

Samples of individual heads of workers were sealed in glass capillaries as described by MORGAN (1990) and subjected to gas chromatography-mass spectrometry using the solventless solid injection technique of MORGAN and WADHAMS (1972), with a Hewlett-Packard 5890 Gas Chromatograph and 5970B Mass Selective Detector with HP59970C ChemStation. Chromatography was carried out on a fused silica capillary column (12 m × 0.2 mm) coated with methyl silicone gum (equivalent to OV-1) of 0.33 m film thickness connected through a 10 m length of deactivated silica tubing (i.d. 0.32 mm) to the source of the mass spectrometer. The capillary tubes were heated in the injector for 2-3 minutes at 140°C before crushing. The injection splitter was closed for the injection and opened 30 seconds later. The carrier gas was helium at 10 psi column head pressure. The oven temperature was at 30°C for 2 min and then increased at 8°C min^{-1} to 250°C. The Mass Selective Detector was set to monitor m/z 35-350 in the scan mode (about 1.5 scans S^{-1}) under Autotune conditions using 70 eV ionization.

4-Methyl-3-heptanol, 4-methyl-3-octanol, and 4-methyl-3-nonanol were synthesized from propanal and 2-bromopentane, 2-bromohexane and 2-bromononane respectively via a Grignard reaction. 4,6-Dimethyl-3-octanol was synthesized via a

crossed aldol condensation between 2-methylbutanal and 3-pentanone (FALES *et al.*, 1980), followed by dehydration to give 4,6-dimethy-4-octen-3-one which was hydrogenated to 4,6-dimethyloctan-3-one and reduced ($NaBH_4$) to 4,6-dimethyloctan-3-ol.

RESULTS AND DISCUSSION

A typical gas chromatogram of a head of *T. caespitum* is shown in Fig. 1. The mass spectra of the three unknown compounds were all similar to that of 4-methyl-3-hexanol and had base peaks at m/z 59 (Table 1), indicating that they were 3-alkanols. On possible biosynthetic grounds, they were thought to be 4-methyl-3-heptanol, 4-methyl-3-octanol and 4-methyl-3-nonanol. The retention times and mass spectra of authentic specimens of the first two confirmed these identifications, but the third compound eluted earlier than 4-methyl-3-nonanol and the two mass spectra did not correspond. Presuming these compounds to be produced from

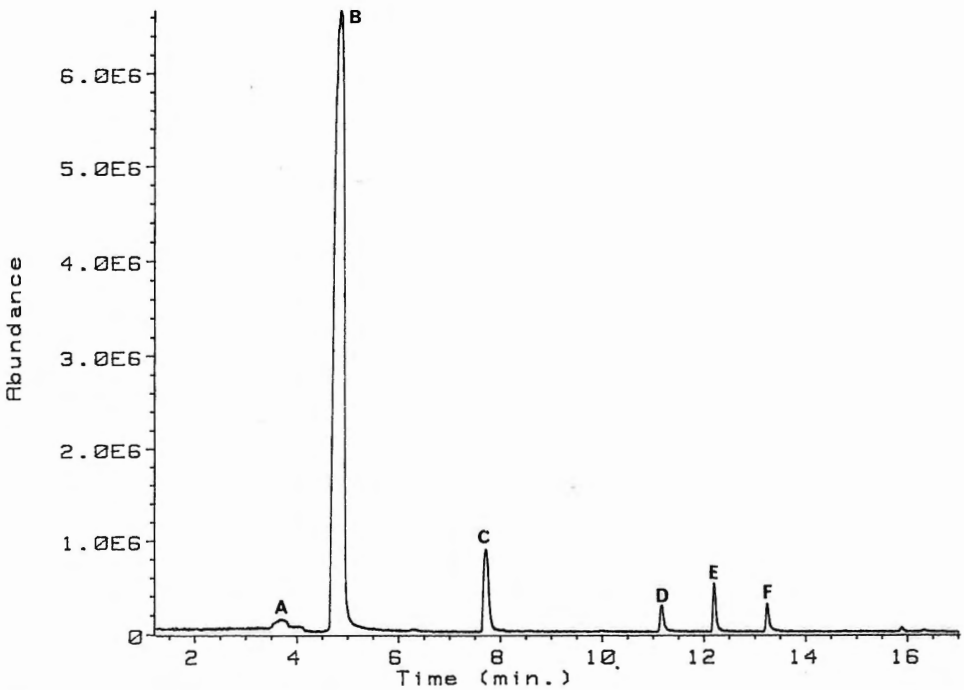

Fig. 1. — Gas chromatogram obtained from four heads of workers of *T. caespitum* without use of solvent, by the solid sampling technique. 2-Pentanone elutes under these conditions with the initial air and water peak (<2 min). The identified peaks are A, 4-methyl-3-hexanone; B, 4-methyl-3-hexanol; C, 4-methyl-3-heptanol; D, 4-methyl-3-octanol; E, nonanal; F, 4,6-dimethyl-3-octanol.

polyketides formed by mixed condensations between malonate (or acetate) and methylmalonate (or propionate) groups, the structure was now presumed to be 4,6-dimethyl-3-octanol. This too was prepared synthetically and shown to be identical in retention and mass spectrum to the mandibular compound. In our earlier paper we had claimed to identify the third minor component as decanal, but we now believe this was wrong and have confirmed, with a synthetic specimen, that it is nonanal.

The mandibular glands of *Tetramorium caespitum* from Western Europe therefore contain, in increasing molecular size, the compounds 2-pentanone, 4-methyl-3-hexanone, 4-methyl-3-hexanol, 4-methyl-3-heptanol, 4-methyl-3-octanol, nonanal and 4,6-dimethyl-3-octanol. Only 4-methyl-3-hexanone and 4-methyl-3-hexanol are found in more than minor amounts. Usually the alcohol is by far the major component, but in occasional individuals from the same nests, there is as much or more of 4-methyl-3-hexanone (ALI *et al.*, 1987).

The carbon skeletons of these compounds are familiar products of ants, other insects and their close relatives. For example, 4-methyl-3-hexanone, 4-methyl-4-hexen-3-one, 4-methyl-4-hepten-3-one, 4,6-dimethyl-4-octen-3-one and 4,6-dimethyl-4-nonen-3-one are defensive secretions of opilionids (EISNER *et al.*, 1978). Manicone, or [(4*E*)-4,6-dimethyloct-4-en-3-one] and its homologues are compounds from the mandibular gland of *Manica rubida* (BESTMANN *et al.*, 1988), and 4-methyl-3-heptanol, is the trail pheromone of the ant *Leptogenys diminuta* (ATTYGALLE *et al.*, 1988). It is also interesting to note that the absolute configuration of the compound in the mandibular glands of *T. impurum* is (3R,4S)-4-methyl-3-hexanol (PASTEELS *et al.*, 1981) and the *L. diminuta* trail pheromone is (3R,4S)-4-methyl-3-heptanol. We have reported the isolation of a series of 3-alkanones, and 3-alkanols from the mandibular glands of *Myrmica* ants (CAMMAERTS *et al.*, 1983 and earlier papers cited therein). These 3-alkanones form a regular series from 3-hexanone to 3-dodecanone, but include 6-methyl-3-octanone.

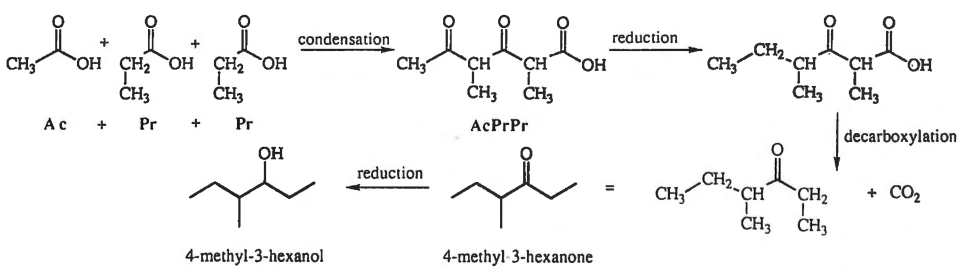

Fig. 2. — The proposed biosynthetic route to the mandibular gland compounds, illustrated for the examples of 4-methyl-3-hexanone and 4-methyl-3-hexanol. For all the compounds found in *T. caespitum* except 2-pentanone, the two right hand units (PrPr) are fixed and variation of the left hand unit (Here Ac), to Pr, AcAc and AcPr gives the other compounds identified.

No biosynthetic studies have been carried out on any of these compounds. It is difficult to see how any of them can arise by simple polyketide synthesis from acetate, but it is possible to propose routes to all of them by simple biosynthetic steps from a combination of acetate (Ac) and propionate (Pr) units (or malonate and methylmalonate), as illustrated in figure 2. The presumed polyketide intermediates can give 2− or 3−alkanones by β-ketoacid decarboxylation. The *T. caespitum* mandibular compounds may be formed in the following ways : 2-pentanone by head-to-tail condensation of three acetate units (AcAcAc) followed by reduction and decarboxylation, 4-methyl-3-hexanol arises from AcPrPr, 4-methyl-3-heptanol from PrPrPr, 4-methyl-3-octanol from AcAcPrPr, and 4,6-dimethyl-3-octanol from AcPrPrPr. Except for 2-pentanone, the final two units of each of these *Tetramorium* compounds are both Pr. The *Myrmica* mandibular gland compounds are postulated to be formed by the following combinations; AcAcPr, PrAcPr, AcAcAcPr, AcPrAcPr, PrAcAcPr, AcAcAcAcPr, PrAcAcAcPr, and AcAcAcAcAcPr. In this group the final two units are always Ac and Pr, once these are fixed, the remainder of the compound appears to be made up of a random mixture of one, two or three acetate or propionate units, to give the various ketones or alcohols.

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PRÉDATION PRÉFÉRENTIELLE DES ADULTES DE *PHYTOSEIULUS PERSIMILIS* SUR CERTAINS STADES DE DÉVELOPPEMENT DE LA PROIE DANS UNE POPULATION DE *TETRANYCHUS URTICAE*

par

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RÉSUMÉ

L'objet de l'étude est de déterminer le stade de développement de *Tetranychus urticae* préférentiellement consommé par le prédateur *Phytoseiulus persimilis* ; à cette fin, des adultes de ce dernier ont été placés dans des colonies de tétranyques qui se sont constituées sur des feuilles de haricot. La structure d'âges de chaque population de tétranyques ayant été étudiée préalablement, le comportement du prédateur lors de son introduction a été suivi, et le stade des première et seconde proies ingérées a été noté. Dans ces conditions, la première proie du prédateur femelle était préférentiellement une femelle adulte de tétranyque, alors que par la suite, lors du deuxième repas, aucune sélection parmi tous les stades de la proie était significativement opérée par le prédateur. Au contraire, les mâles adultes du prédateur ont consommé préférentiellement moins de proies femelles et plus d'œufs. Apparemment, la rencontre de la proie et du prédateur n'est pas due au hasard, mais pourrait impliquer des kairomones.

Mots-clefs : Tetranychus urticae, Phytoseiulus persimilis, prédation, stade de développement.

Preferential predation of *Phytoseiulus persimilis* adults on some developmental stages of the prey within a population of *Tetranychus urticae*

SUMMARY

In order to determine the stage of *Tetranychus urticae* preferentially eaten by *Phytoseiulus persimilis,* adult predators were placed in prey colonies formed on bean leaves. The age struc-

ture of each prey colony had been previously recorded. The behaviour of the predators was then followed and the first and second prey stages eaten by the predator were noted. In these conditions, the first stage eaten by the adult female predator was preferentially a female, while no significant selective predacious behaviour was noted at the second food intake. On the contrary, prey females were less eaten than expected by a random choice by male predators which preferred eggs. Besides, it seems that the prey selection behaviour may involve kairomones.

Keywords : Tetranychus urticae, Phytoseiulus persimilis, predation, development stage.

INTRODUCTION

L'acarien prédateur *Phytoseiulus persimilis* ATHIAS-HENRIOT est un auxiliaire efficace dans le contrôle biologique des acariens phytophages (*Tetranychus urticae* KOCH) particulièrement dans les cultures maraîchères en serre (FOURNIER *et al.*, 1985 ; MORIN, 1988).

Les lâchers de *P. persimilis* s'effectuent de façon curative, là où les premiers dégâts de tétranyques sont observés. Ce prédateur présente la particularité d'avoir une alimentation très spécifique ; en effet, on ne lui connaît comme source de nourriture que des acariens phytophages du genre *Tetranychus,* à de rares exceptions près, comme *Phytonemus pallidus* LINDQUIST, un autre acarien phytophage (SIMMONDS, 1970). En présence de faibles populations de la proie, une telle spécificité alimentaire provoque une diminution marquée des effectifs du prédateur, pouvant même entraîner sa disparition. Dès lors, des introductions répétées du prédateur sont nécessaires à chaque recrudescence des acariens phytophages.

Dans le but de mieux connaître le comportement des *P. persimilis* adultes lors de chaque lâcher, cette étude a porté sur le choix alimentaire de ce prédateur *in situ,* lors de la première et deuxième prise alimentaire. Ainsi, les expériences sont réalisées sur des feuilles préalablement infestées par l'acarien phytophage et comportant tous les stades de développement de la proie, alors que les diverses études antérieures n'envisageaient qu'un ou deux stades de la proie et étaient réalisées dans des conditions plus ou moins artificielles.

MATÉRIEL ET MÉTHODES

L' acarien phytophage *T. urticae,* souche « White Eye » (provenant du Laboratoire d'Entomologie Appliquée de l'Université d'Amsterdam) est élevé dans un phytotron sur haricot (*Phaseolus vulgaris* L.), variété Flotille (Pannevis). Les conditions de température et de luminosité sont de 24 ± 1 °C et de 16 heures d'éclairement par 24 heures (le rayonnement photosynthétiquement actif atteint 80 microeinsteins m^{-2} s^{-1} à 10 cm au-dessus des plants). L'humidité relative y est de 55 ± 5 %.

Dans la chambre d'élevage du tétranyque, des disques d'un diamètre de 2,4 cm sont découpés dans des feuilles de haricot sur lesquelles la population du ravageur présente tous les stades de développement. Les disques de feuille sont prélevés au nombre de 50 et sont déposés dans des cellules de Münger, face inférieure vers le

dessus, sur un papier filtre humidifié. Le papier filtre fortement humidifié maintient la feuille de haricot dans un état hydrique satisfaisant. Tous les stades de développement du tétranyque sont recensés sous binoculaire.

Un individu adulte du prédateur, *P. persimilis,* en phase d'activité sexuelle (de 3 à 6 jours de vie adulte) et de souche provenant de la firme Koppert (Pays-Bas) est déposé à l'aide d'un pinceau, à jeun, au centre de chacun de ces disques. Sous binoculaire, à température de 22 ± 2°C, le comportement du prédateur est suivi et le stade de la première, puis de la seconde proie attaquées avec succès par le prédateur est noté dans une des catégories suivantes : œuf, larve, nymphochrysalide, protonymphe, deutochrysalide, deutonymphe, téléochrysalide, adultes mâle et femelle. Lors de l'observation, les papiers filtres sont régulièrement réhumidifiés afin de maintenir une humidité relative constante au niveau des feuilles de haricots. Sur chaque rondelle de feuille, l'expérience est répétée 5 fois respectivement avec des prédateurs mâles et femelles, après le remplacement de chaque proie prélevée. Le nombre de répétitions pour chaque sexe du prédateur et pour chaque type de repas (1er ou 2^{e}) est donc de 5 × 50, soit 250 prises de nourriture.

TABLEAU 1

Nombres (observés et attendus) des stades de Tetranychus urticae *tués par les femelles de* Phytoseiulus persimilis.

	Stade de la proie tué								
	o	l	nc	pn	dc	dn	tc	m	f
1er repas									
Nbre observé	175,0	4,0	3,0	6,0	1,0	5,0	4,0	10.0	42,0
Nbre attendu	192,0	8,9	3,4	4,6	4,1	5,1	4,0	8,1	19,8
Différence	−17,0	−4,9	−0,4	1,4	−3,1	−0,1	0	1,8	22,2
Chi-carré	1,5	2,7	0	0,4	2,3	0	0	0,4	25,0**
2^{e} repas									
Nbre observé	196,0	8,0	4,0	3,0	0	3,0	1,0	10,0	25,0
Nbre attendu	192,0	8,9	3,4	4,6	4,1	5,1	4,0	8,1	19,8
Différence	4,0	−0,9	0,6	−1,6	−4,1	−2,1	−3,0	1,9	5,2
Chi-carré	0,1	0,1	0,1	0,5	4,1	0,9	2,3	0,4	1,4

Stades du tétranyque : o : oeuf ; l : larve ; nc : nymphochrysalide ; pn : protonymphe ; dc : deutochrysalide ; dn : deutonymphe ; tc : téléochrysalide ; m : mâle adulte ; f : femelle adulte.
** : $p < 0,01$.

TABLEAU 2

Nombres (observés et attendus) des stades de Tetranychus urticae *tués par les mâles de* Phytoseiulus persimilis.

	Stade de la proie tué								
	o	l	nc	pn	dc	dn	tc	m	f
1er repas									
Nbre observé	200,0	4,0	0	3,0	2,0	6,0	1,0	10.0	21,0
Nbre attendu	192,0	8,9	3,4	4,6	4,1	5,1	4,0	8,1	19,8
Différence	8,0	−4,9	−3,4	−1,6	−2,1	0,9	−3,0	1,9	1,2
Chi-carré	0,3	2,7	3,4	0,5	1,1	0,1	2,3	0,4	0,1
2e repas									
Nbre observé	221,0	4,0	0	3,0	2	3,0	2,0	7,0	8,0
Nbre attendu	192,0	8,9	3,4	4,6	4,1	5,1	4,0	8,1	19,8
Différence	29,0	−4,9	−3,4	−1,6	−2,1	−2,1	−2,0	−1,1	−11,8
Chi-carré	4,4*	2,7	3,4	0,5	1,1	0,9	1,0	0,1	7,0**

Stades du tétranyque : o : oeuf ; l : larve ; nc : nymphochrysalide ; pn : protonymphe ; dc : deutochrysalide ; dn : deutonymphe ; tc : téléochrysalide ; m : mâle adulte ; f : femelle adulte. * : $p<0,05$; ** : $p<0,01$.

Un test de Chi-carré permet de comparer, au niveau de chaque stade de *T. urticae,* le nombre attendu de proies prélevées au nombre observé de prélèvements. La valeur attendue se base sur l'hypothèse d'une prise aléatoire de la proie. Elle est calculée en multipliant le nombre total de prises de proie (250) par l'abondance relative de chaque stade de la proie. Pour le cas où des petits échantillons sont traités, la correction suivante a été effectuée : dans le cas où la valeur de Chi-carré obtenue est en-dessous du seuil de signification, la différence est jugée non significative ; par contre, si la valeur de Chi-carré égale ou dépasse ce seuil, la correction de Yates est appliquée en soustrayant 0,5 de la différence (valeur absolue) entre les valeurs attendue et observée (SCHWARTZ, 1963).

RÉSULTATS

Prédation exercée par les femelles de *Phytoseiulus persimilis*

Les femelles de *P. persimilis* ne tuent pas leur première proie de manière aléatoire au sein d'une population de tétranyques (Tableau 1). En effet le test statistique révèle que les femelles adultes de la proie sont prélevées en plus grand nombre,

comparativement à une prédation due au hasard. Il n'en est pas de même pour les autres stades du tétranyque qui, eux, sont prélevés aléatoirement. Dès le deuxième repas, aucune préférence pour un stade donné de la proie n'apparaît, y compris vis-à-vis des femelles adultes.

Prédation exercée par les mâles de *Phytoseiulus persimilis*

A l'opposé des prédateurs femelles, les prédateurs mâles exercent, lors du premier repas, un prélèvement aléatoire parmi les stades de développement possibles de leur proie (Tableaux 1 et 2). En revanche, un prélèvement préférentiel apparaît au cours du deuxième repas du prédateur mâle, aux dépens du stade œuf de *T. urticae,* alors que les femelles adultes sont significativement moins attaquées. Ceci pourraît être attribué à une taille plus faible du prédateur mâle ; en effet, celui-ci se trouverait limité dans ses capacités de prédation à l'égard de proies plus grandes, plus difficiles à capturer (SABELIS, 1981).

Cet aspect non-aléatoire du choix du stade de la proie par le prédateur adulte constitue un élément explicatif de la déstructuration que provoque le prédateur au sein de la population du tétranyque (HANCE et PASLEAU, 1987).

DISCUSSION

Au sein d'une colonie de tétranyques, l'ingestion d'une proie par le prédateur *P. persimilis* peut être considérée comme la conséquence de deux processus préalables se succédant dans le temps (SABELIS, 1981). Tout d'abord, la rencontre entre le prédateur et la proie et, ensuite, la reconnaissance de celle-ci comme proie. Elle dépend donc à la fois du taux de rencontre entre le prédateur et la proie, et du taux de réussite de la rencontre, deux facteurs qui sont propres à chaque stade.

La localisation de la proie a longtemps été considérée comme une question de « chance » (MORI et CHANT, 1966). Ce n'est que récemment qu'il a été démontré que certains Phytoseiidae sont sensibles à des substances chimiques émises par leurs proies, qui agissent comme kairomones d'attraction pour la localisation de colonies éloignées de tétranyques (SABELIS et VAN DE BAAN, 1983 ; SABELIS *et al.,* 1984). Cependant, SABELIS (1981) considère que dans la zône de la feuille infestée par l'acarien phytophage, la localisation de la proie par le prédateur n'est probablement qu'une question de chance. Dans ses expériences, cet auteur ne trouve que de faibles différences entre le taux de contact proie-prédateur et celui calculé à partir d'un modèle de recherche aléatoire.

Dans nos conditions expérimentales, les nombres de rencontres proie-prédateur peuvent être calculés à partir des nombres de proies prélevées, sur la base du taux de réussite. Celui-ci exprime le pourcentage de contact se terminant effectivement par l'ingestion de la proie. Le taux de réussite est propre à chaque stade de la proie, les capacités de fuite et la masse de la proie étant deux facteurs importants de variabilité selon SABELIS (1981), lequel a déterminé le taux de réussite pour les contacts entre la femelle de *P. persimilis* et les différents stades de sa proie. Sur base du

calcul incluant les données de cet auteur, il s'avère que la rencontre entre le *P. persimilis* à jeun et la femelle ou le mâle de *T. urticae* n'est pas aléatoire, dans nos conditions expérimentales, au sein d'une colonie de tétranyques ($p < 0,01$ et 0,05 respectivement, aussi bien au cours du premier que du deuxième repas).

Dans le calcul du nombre attendu de rencontres proie-prédateur, nous n'avons pu tenir compte des facteurs intervenant dans la rencontre purement liée au hasard : la taille de l'individu (proie et prédateur), l'activité (% du temps de l'acarien consacré à son déplacement) et la vitesse de déplacement. Les différences de taux de rencontre relatif (taux de rencontre rapporté à la densité de la proie), sur la feuille et en présence de toile, entre une femelle de *P. persimilis* et un stade du tétranyque nous apparaissent cependant peu significatives en fonction du stade de la proie : lorsque celle-ci est une femelle mobile, SABELIS (1981) relève une valeur calculée et observée de 0,18 et 0,17 contacts.min.$^{-1}$ et, lorsque la proie est un œuf, de 0,12 et 0,14 contacts.min.$^{-1}$.

La raison de la rencontre significativement plus importante entre la femelle du prédateur et les adultes de la proie n'est pas aisée à déterminer. JACKSON et FORD (1973) émettaient l'hypothèse selon laquelle *P. persimilis* entrerait en contact avec sa proie de manière fortuite, et que des chémorécepteurs de contact lui permettraient d'identifier alors la proie. Notre expérience ne permet pas de conforter cette idée au vu du faible pourcentage d'adultes (5 %) dans les populations denses de tétranyques (moyenne de 210 individus dm^{-2}, tous stades confondus) qui sont mises en présence du prédateur. Plus probablement, des kairomones agissant sur des faibles distances à partir de leurs points d'émission pourraient être impliquées dans le choix préférentiel du stade de la proie au sein de la colonie. Il n'est pas établi que les kairomones volatiles déjà mises en évidence soient identiques à celles qui provoqueraient le choix préférentiel d'un stade de la proie au sein d'une colonie. De plus, ce type d'action intermédiaire entre celle des kairomones de contact et volatiles pourrait expliquer les conclusions de SABELIS (1981), lequel a travaillé en conditions, de faible densité de la proie et avec des colonies de la proie exclusivement composées d'un stade.

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SPIDERS OF THE FAMILY HAHNIIDAE FROM SULAWESI, INDONESIA WITH REMARKS ON SYNONYMY AND ZOOGEOGRAPHY (ARACHNIDA : ARANEAE : HAHNIIDAE)

by

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SUMMARY

The author describes a new *Hahnia* and a new *Alistra* (Araneae : Hahniidae), collected during the Alfred Russel Wallace Commemorative Expedition to the Dumoga-Bone National Park in Sulawesi Utara, Indonesia. The synonymy of *Scotussa* SIMON, 1898 and *Muizenbergia* HEWITT, 1915 with *Hahnia* C.L. KOCH, 1841 is confirmed.

Keywords : Hahniidae, systematics, zoogeography, Sulawesi Utara.

INTRODUCTION

« Project Wallace », a major expedition to Sulawesi Utara (North Celebes, Indonesia), was organised by the Royal Entomological Society of London to commemorate its 150th anniversary and the centenary of its Royal Charter. Alfred Russel WALLACE was a former fellow of the Royal Entomological Society and his famous work in the Indo-Australasian Region is immortalised in the use of the name "Wallacea" for the faunal transition zone between Australasia and Asia. The forests of this region are extremely rich in species and those of Sulawesi are of particular interest because of the high levels of endemicity for which the island is famous.

The expedition occupied the whole of 1985; the author participated during October and November. The expedition's base camp was situated in the Dumoga-Bone National Park just west of Kotamobagu. This is a large region of unspoilt rain forest ranging in altitude from 200 to 1800 m.

Among the rich spider fauna in the forest, species of the family Hahniidae were common especially in the litter layer, as were the Zodariidae, treated in a previous paper (BOSMANS and HILLYARD, 1990).

MATERIAL AND METHODS

Spiders were collected by pitfall trapping, sieving litter, sweeping vegetation and hand collecting.

Descriptions of species are as in BOSMANS and THIJS (1980). Holotypes are deposited in the « Koninklijk Belgisch Instituut voor Natuurwetenschappen » (K.B.I.N.) in Brussels ; paratypes in the same institute, in the « Muséum national d'Histoire naturelle de Paris » (M.N.H.N.P.), and in the authors private collection (C.R.B.).

Abbreviations used in the descriptions are : Fe, Pa, Ti, Mt, Ta : Femur, patella, tibia, metatarsus and tarsus. AM, AL, PM, PL : anterior median, anterior lateral, posterior median and posterior lateral eyes.

Measurements are in mm.

DESCRIPTION OF SPECIES

Hahnia barbata sp. n.

(Fig. 1, 1-6)

Diagnosis :

Males of *Hahnia barbata* are easily distinguished by the row of bristles of the bulbus op the palp ; females are less readily distinguished by details in the vulva : the secondary receptacula larger than the primary, and the presence of four loops in the chitinous part of the copulation ducts.

Etymology :

The name refers to the bristles on the bulbus of the male palp.

Type material :

Holotype male : Indonesia, Sulawesi Utara, Dumoga-Bone national Park, trail near river Toraut (« 1440 trail ») :

— 400 m, pitfall in natural lowland rainforest, 5.XI.1985 ; deposited in K.B.I.N.

Paratypes : 10 males 2 females, same locality and date (K.B.I.N.) ;

— 200 m, 2 males 2 females in forest litter near river Toraut, 24.X.1985 (M.N.H.N.P.) ;

— 300 m, 2 males 2 females in pitfalls in rain forest, 24.X.1985 (C.R.B) ;

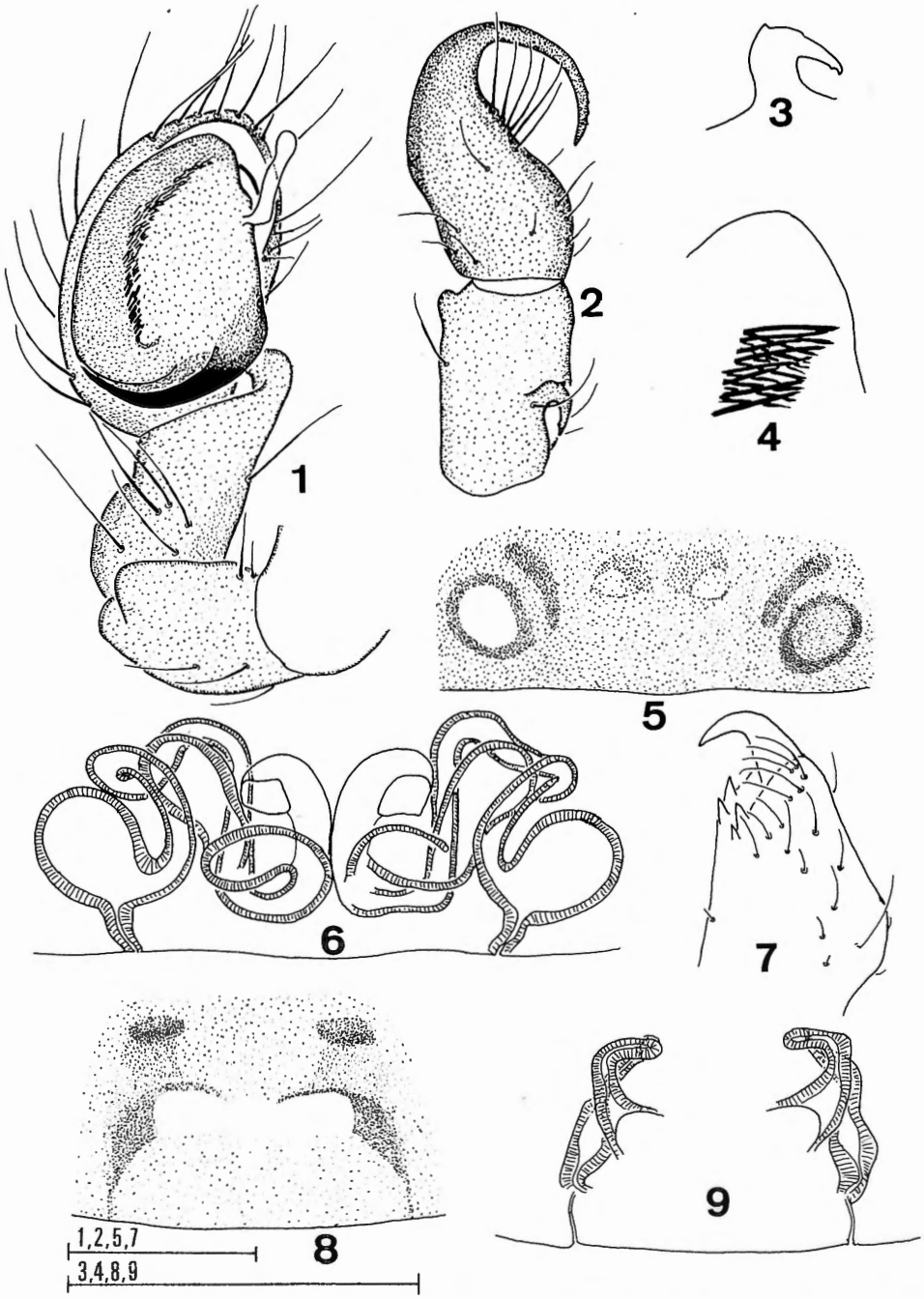

Fig. 1. — 1-6. *Hahnia barbata* sp. n. 1. Male palp, ventral view ; 2. Femur and tibia of the male palp, lateral view ; 3. Apophysis of male palpal femur, lateral view ; 4. Detail of bristles on male palpal cymbium, dorso-lateral view. 5. Epigyne. 6. Vulva. Scale line : 0.2 mm. — 7-9. *Alistra sulawesensis* sp. n. 7. Right chelicera, frontal view ; 8. Epigyne. 9. Vulva. Scale line : 0.2 mm.

— 660 m, 4 males 1 female in pitfall in rain forest, 24.X.1985 (C.R.B.);
— 800 m, 4 males in pitfalls in rain forest, 24.X.1985 (C.R.B.).

Description :

Male holotype : Measurements : Total length 2.16 (1.96-2.32); prosoma 1.20 (0.96-1.20) long, 0.80 (0.96-1.20) wide; chelicerae 0.50 long; sternum 0.67 long, 0.64 wide.

Colour : Prosoma greyish to chocolate brown, with darkened striae, fovea and margin, and wide submarginal yellowish-brown stripe. Chelicerae yellowish brown suffused with grey. Sternum yellowish brown with darkened margin. Legs yellowish brown, Fe, Ti and Mt each with two greyish-brown annulations. Abdomen dorsally grey to dark grey, with 5 pairs of oblique chevrons, the posteriors generally interconnected; ventrally paler in the middle. Outer and intermediate spinnerets greyish brown with pale yellowish base and tip, inner spinnerets yellowish brown.

Prosoma : With shallow medio-dorsal concavity in lateral view. Clypeus as wide as the diameter of the AM. Eyes closely set, all separated by less than their radius. Median ocular quadrangle 0.75 × wider posteriorly than anteriorly, and 0.9 × as wide as long.

Chelicerae : With three anterior and 6 posterior teeth in fang groove; stridulating file indistinct.

Legs : Fe with 1 dorsal spine, Fe I with an additional prolateral spine. Ti I-II with 2 dorsal and 1 prolateral spine, Ti III-IV with two additional ventral spines. Measurements (holotype) :

	Fe	Pa	Ti	Mt	Ta
I	0.84	0.36	0.71	0.69	0.50
IV	0.93	0.34	0.72	0.81	0.58

Abdomen : Spiraculum closer to the epigastric furrow than to the spinnerets (ratio : distance epigastric furrow — spiraculum to distance epigastric furrow — spinnerets equals 0.31). Spinnerets on a slightly curved row, distal segment of outer spinnerets slightly shorter than basal one.

Palp (Fig. 1, 1-4) : Femur with hooked baso-lateral apophysis, provided with two denticules, and lobed antero-mesal apophysis. Tibia elongate, longer than wide, with long, semi-circular spur, gradually narrowing and with minute denticules. Cymbium rounded anteriorly, not much longer than bulbus. Bulbus oval, with a distinct curved row of bristles; embolus encircling the bulbus, reaching slightly further than the membranous conductor.

Female : Measurements : Total length 2.36-2.76; carapace 1.10-1.29 long, 0.88-0.94 wide.

Colour and general appearance as in the male.

Epigyne (Fig. 1, 5) : Without outer chitinous structures. Generally rather dark, with ducts or spermathecae hardly shining through ; in paler specimens the secondary spermathecae and one oblique duct are visible.

Vulva (Fig. 1, 6) : Copulation openings situated in the antero-median part of the epigyne. Non-chitinous entrance duct short, consisting of only one loop ; bifurcation point connected by one loop of the chitinous copulation duct to the oval primary receptacula, and by four loops to the rounded and larger secondary receptacula.

Distribution and ecology :

Only known from Sulawesi Utara, where it is common in litter of lowland forests. It occurred up to 800 meters, and was absent from samples taken at higher altitudes.

Remarks :

The row of bristles on the male bulbus is very peculiar. Besides in this species, it is only known to occur in the European *H. ononidum* SIMON, but in this species it is much shorter and even difficult to perceive. A male *Hahnia* from Sumatra sent to me by C. Deeleman-Reinhold shows however a similar row of even longer bristles than in *H. barbata* sp. n.

Alistra sulawesensis sp. n.

(Fig. 1, 7-9)

Diagnosis :

This species differs from other *Alistra* species by the contrasting colour pattern of the abdomen and by the vulva with reduced primary receptacula and elongate secondary receptacula.

Etymology :

The species is named after the type locality.

Type material :

Holotype female : Indonesia, Sulawesi Utara, Dumoga-Bone National Park, Lake Mooat, 1100 m, plantation, 28.X.1985 ; deposited in K.B.I.N.

Description :

Female : Measurements : Total length 2.38 ; prosoma 0.98 long, 0.76 wide.

Colour : Prosoma reddish brown, margin and striae greyish ; chelicerae yellowish brown with grey spots ; sternum yellowish brown suffused with grey ; legs yellowish brown, tibiae with two greyish annulations, femora with traces of annulations ; abdomen grey, a broad lateral band, dorsally encircling spinnerets and pedicel, is dark grey speckled with pale grey. Spinnerets yellowish white, except for the greyish-black distal segments of the outer spinnerets.

Prosoma : Pear-shaped. Clypeus slightly wider than the diameter of the AM. Eyes equal, AM separated by 2/3 their diameter, from the AL by their radius ; PM separated by 1.5 their diameter, from the PL by 0.95 their diameter.

Chelicerae (Fig. 1, 7) : With anterior boss, and with 2 anterior and 2 posterior teeth in fang groove. No stridulating file observed.

Legs : With very long hairs, often difficult to distinguish from spines. Fe I with one prolateral spine ; Ti with 1 dorsal spine. Measurements :

	Fe	Pa	Ti	Mt	Ta
I	0.88	0.30	0.70	0.57	0.48
IV	0.96	0.28	0.90	0.54	0.52

Abdomen : Spiraculum situated closer to the spinnerets than to the epigastric furrow (ratio : distance epigastric furrow — spiraculum to distance epigastric furrow — spinnerets equals 0.66). Spinnerets on a slightly curved row, very long ; measurements : outer spinneret : 0.74, with segments respectively 0.35, 0.19 and 0.24 long ; intermediate : 0.36 ; inner : 0.30.

Epigyne (Fig 1, 8) : Without external chitinisations, representing a median transverse pale spot, flanked by two darkened oblique stripes, widened in the middle.

Vulva (Fig. 1, 9) : Copulation openings situated in the medio- lateral part of the epigyne, connected by a short, anteriorly directed chitinised duct to the hardly thickened primary receptacula, then by a posteriorly directed duct to the elongate secondary receptacula.

Distribution :

Only known from the type locality.

DISCUSSION

The Hahniidae collected in the Dumoga Bone National Park belong to the genera *Hahnia* and *Alistra*.

Species from all over the world have been placed in the genus *Hahnia*. As many of the exotic species were placed in the genus only according to the relative size of

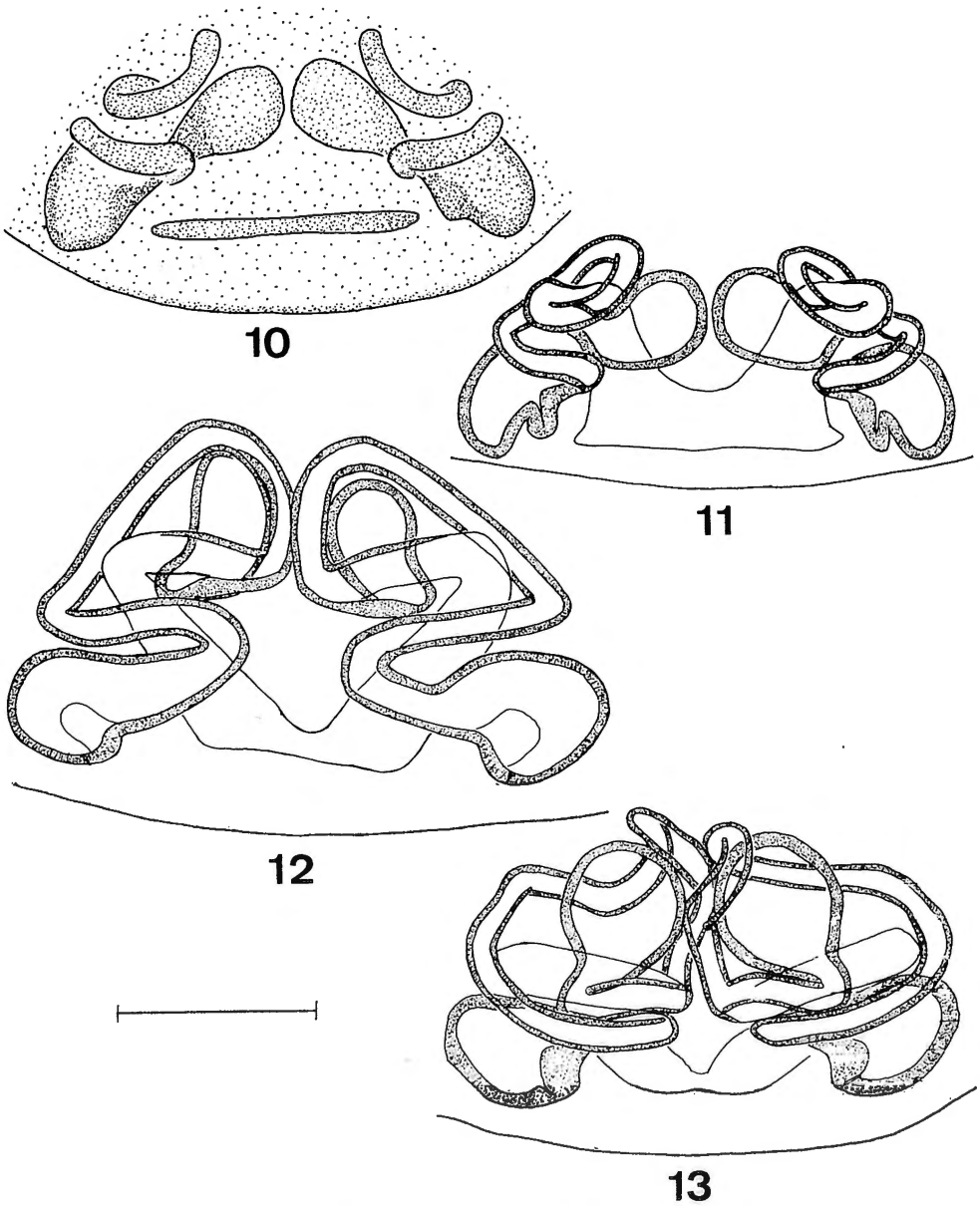

Fig. 2. — 10-11. *Hahnia zodarioides* (SIMON). 10. Epigyne, ventral view ; 11. Vulva, ventral view. — 12. *Hahnia abrahami* (HEWITT) : vulva, ventral view. — 13. *Hahnia eidmanni* (ROEWER) : vulva, ventral view. Scale line : 0.1 mm.

the anterior median eyes, and drawings of the genital organs of these exotic species were never published, this has to be completely reconsidered. LEHTINEN (1967)

already transferred some species to other genera, and limited the distribution area of *Hahnia* to the holarctic-neotropical region. But to my knowledge, not one of the neotropical species is adequately illustrated to prove its generic position, and the presence in this region of *Hahnia* remains to be confirmed.

LETHINEN (1967) claimed the afrotropical genus *Muizenbergia* HEWITT, 1915 to be a distinct genus and considered *Scotussa* SIMON 1898 and *Hahniops* ROEWER, 1947 as junior synonyms. Apart from the fact that *Scotussa* SIMON, 1898 is the senior synonym and should have priority over *Muizenbergia* HEWITT, 1915, we disagree with LEHTINEN'S point of view. The following (type) specimens were examined :

— 1 male 1 female of *Hahnia pusilla* C. L. KOCH, Houthulst, Belgium (type species of *Hahnia* ; C.R.B.) ;

— Holotype female of *Scotussa zodarioides* SIMON, Cape of Good Hope, (M.N.H.N.P. 18560) ;

— One female of *Muizenbergia abrahami* HEWITT (Natal Museum, Pietermaritzburg) ;

— Holotype female of *Hahniops eidmanni* ROEWER, Fernando Poo, Pic Isabel (Forschungsinstitut Senckenberg, Frankfurt am Main).

Fig. 2 (10-13) shows epigyne and/or vulva of *Scotussa zodarioides* (illustrated for the first time here), *Muizenbergia abrahami* and *Hahniops eidmanni*. All show the typical copulatory openings, entrance ducts, connecting ducts, primary and secondary receptacula, all present in the type species of *Hahnia* as well. We consider them all as valid *Hahnia* species, and the three genera are therefore considered synonyms of Hahnia. This was already confirmed by me for the genus *Hahniops* (BOSMANS 1980), but not yet for *Muizenbergia* and *Scotussa*. Subsequently, we described several new species from tropical Africa in the genus *Hahnia* (BOSMANS and THIJS 1980, BOSMANS 1981, 1982a-b, 1986, 1987, JOCQUÉ and BOSMANS 1982). A part of this point of view was recently and indepently confirmed by LEDOUX (1991). After having studied the species *Hahnia crozetensis* HICKMANN from the Island Crozet, LEDOUX synonymised *Muizenbergia* with *Hahnia*. In this paper we further confirm the synonymy of *Scotussa* with *Hahnia*.

The genus *Hahnia* thus has most probably a holarctic-afrotropical distribution, and *Hahnia barbata* sp. n. represents a palaeotropical element of the fauna of Sulawesi.

The genus *Alistra*, THORELL 1894 on the other hand has according to LEHTINEN (1967) an oriental-australian distribution. He considered *Aviola* SIMON, 1898, *Bigois* SIMON, 1898 and *Nanonymphaea* RAINBOW, 1920 as junior synonyms. The genus is mainly diagnosed by the simple genital organs : short patellar and tibial apophyses in the male palp ; short entrance and connecting ducts and reduced primary receptacula in the female vulva. *Alistra sulawesensis* sp. n. represents an indo-australian element of the fauna of Sulawesi.

So far, two Hahniidae are known to occur in Sulawesi Utara : *Hahnia barbata* sp. n., a palaearctic-afrotropical element, and *Alistra sulawesensis* sp. n., an indo-

australian element. Once more, the diversity of the fauna of Sulawesi and the mixture of elements from different zoogeographical regions is proved.

Whereas *Hahnia* species inhabit the litter layer of forests and bushes, *Alistra* species seem to live on leaves and branches of trees. This was indicated by BRIGNOLI (1986) is his description of *Alistra mendanai* from the Solomon islands, in the present paper for *Alistra sulawesenis* sp. n., and by Dr. DEELEMAN-REINHOLD *in litteris*.

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(Manuscript received on 12 March 1991)

FOOD AND FEEDING HABITS OF *HAPLOCHROMIS* (TELEOSTEI : CICHLIDAE) FROM LAKE KIVU (CENTRAL AFRICA)

II. Daily feeding periodicity and dietary changes of some *Haplochromis* species under natural conditions

by

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SUMMARY

The feeding periodicity and dietary changes of *Haplochromis graueri, H. olivaceus, H. nigroides* and *H. kamiranzovu,* 4 endemic species from lake Kivu, were investigated by determining the stomach fullness index at intervals of 3 hours over a 24-hour cycle. These 4 species showed 2 types of daily feeding activity rhythm both characterised by a major food intake at daytime. A first type of bimodal feeding rhythm with the first peak situated in the morning between 6.00 a.m. and 12.00 a.m. and a second one in the evening between 3.00 and 9.00 p.m. characterizes *H. graueri,* and *H. olivaceus. H. kamiranzovu* and *H. nigroides* on the other hand showed a unimodal feeding rhythm with a single peak situated in the afternoon between 3.00 and 6.00 p.m.

The beginning of each period of active foraging is rather easily detected in catches by a rapid rise in number of fish captured. The highest degree of stomach fullness appears almost invariably about 3 hours after the beginning of such a foraging peak.

The variation of the coefficient of vacuity in the various *Haplochromis* species studied is not only related to the dial feeding rhythm but also to the items ingested : it is higher in carnivorous species and lower in omnivorous, detritivorous and phytophagous ones.

The relation between the uptake of various prey types and feeding time over the 24 h period indicates that some prey items are more accessible at specific hours of the day. These *Haplochromis* species have developed a strategy for cropping potential food items at specific periods of the day. This probably leads to a further reduction or in some cases to a complete absence of interspecific food competition.

Keywords : Feeding periodicity, food and feeding habits, *Haplochromis,* Cichlidae, lake Kivu.

INTRODUCTION

Haplochromis is one of the largest and most widespread genera of cichlids in lake Kivu (Central Africa). The genus is represented by at least 13 species which are all endemic to the lake. In 1977, attention was drawn to their ecology and several field missions have been organized by the « Musée Royal de l'Afrique Centrale » (MRAC, Tervuren-Belgium) in collaboration with the « Institut National de la Recherche Scientifique » (INRS, Butare-Rwanda) in order to investigate the biology of various species of *Haplochromis*. Several aspects of their biology are under investigation (systematics, reproduction, growth, food and feeding habits) and several papers have been recently published on their systematics (COENEN *et al.*, 1984 ; SNOEKS, 1986, 1988 ; SNOEKS *et al.*, 1984, 1987 and 1990) while only a few on their food and feeding ecology (VERBEKE, 1957, *Ulyel et al.*, 1990).

Although it is known that fish species differ widely in their food and daily feeding pattern, these aspects have received little attention in the *Haplochromis species of lake Kivu*. THYS VAN DEN AUDENAERDE (1986) observed that *Haplochromis* species from lake Kivu were active during the day, but asleep at night. DE VOS *et al.* (1987) concluded that some *Haplochromis* species such as *Haplochromis kamiranzovu* showed vertical food migration during the night while some others species like *H. graueri, H. gracilior* seemed to be strictly benthic and inactive.

Those studies did not take into account, even partially, the food, feeding habits, nor stomach fullness which are the most important ways to investigate food availability and abundance, ecological separation of fish assemblages and the utilisation of food under natural conditions.

The present paper deals with the daily feeding periodicity of 4 *Haplochromis* species. The ecological significance of the diet in relation to different kinds of food items eaten during the 24-hour period is also discussed.

MATERIAL AND METHODS

Fieldwork was carried out at Gisenyi and Kibuye Bay in lake Kivu (Rwanda, 1.500 m altitude), one of the smallest lakes of the East-African Rift Valley with a total water surface of about 2.370 km^2 and with a mean depth of 240 m. Lake Kivu (Fig. 1) is located south of the Equator (1°34'30-2°30' Latitude South and 28°50'-29°23 Longitude East). It is surrounded by several active volcanoes (Nyamulagira, Nyiragongo...) and geothermal springs. It is characterized by a very high concentration of dissolved gas (CH_4, 370 ml/l ; CO_2, 1400 ml/l) in the deeper layers. The temperature of water at the surface ranges between 24 to 25.5 °C (DEGENS *et al*, 1973 ; DEUSER *et al.*, 1973 ; DE JONGH and SPLIETHOFF, 1980).

The study was based on six 24-hour periods of capture at intervals of 3 hours on August 18-31, 1981 (Murakoze mission IV) and on February 12-17, 1987.

Fishing periods cover the whole interval of 3 hours and were given as the time the nets were emptied (*e.g.* the 9.00 a.m. sample covered 6.00 to 9.00 a.m. fishing).

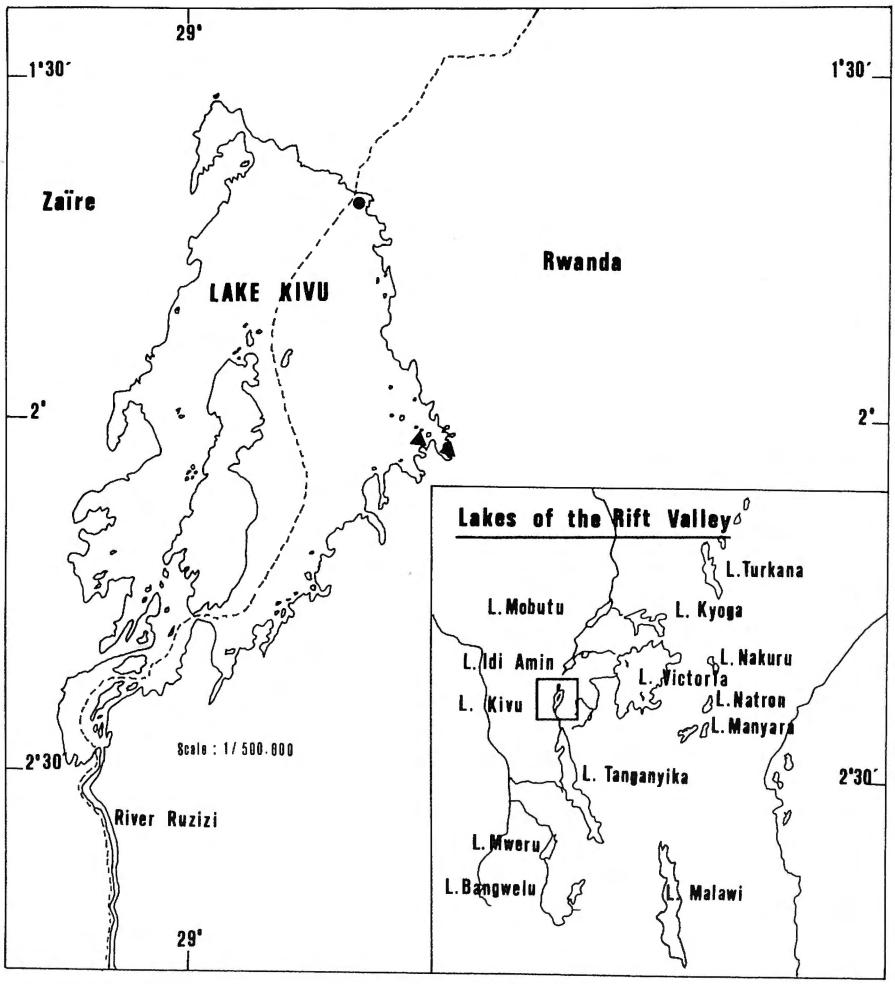

Fig. 1. — Map of lake Kivu showing geographical location and study area. Fishing stations — (▲) Kibuye bay ; (●) Gisenyi bay.

Fish were collected at two sites using monofilament bottom gill-nets of :

(a) 15-10-40-30-20-20-12 and 10 mm ktk,

(b) 12-15-12-40-30-20-20 and 15 mm ktk mesh-size at Kibuye Bay between 0 and 70 m depth (see THYS VAN DEN AUDENAERDE, 1986) and bottom gill-nets of 10-12-15-20 and 25 mm mesh-size at Gisenyi Bay (0-45 m depth).

A 24-hours experimental fishing protocol was organized at Kibuye Bay by the Murakoze IV group.

A first series (a) of gill-nets was first placed in the water at 6.00 a.m. ; at 9.00 a.m., series (b) was set at some distance, and 3 hours later on, the set of net

(a) was taken out of the water. At 12.00 a.m., set (b) was taken out and the series of the net (a) was set again but at some distance with a switching of the two first nets to the end of the series and so on. In this way, each net of each different mesh-size was fishing at various depths and during one or more of various periods of the day (THYS VAN DEN AUDENAERDE, 1986).

After the capture, the fishes were immediately injected intraperitoneally with 10 % buffered formaldehyde in order to stop digestion, and later on, they were transferred to 70 % ethanol to preserve gut contents for subsequent analysis. In the laboratory, the stomach and gut contents were removed and kept separately in microtubes in 70 % ethanol. Only stomach contents were examined for the purpose of this study.

For the estimation of the « fullness index » and the quantification of each food item, the numerical method and point methods (HYNES, 1950 ; WINDELL, 1968 ; HYSLOP, 1980) were used. A defined number of points depending on the visually estimated volume was awarded to each stomach content and food item. Although the point method (HYNES 1950 ; WINDEL, 1968) is considered to be a « subjective allotment of points », it has the advantage of being easy, fast and adapted to any kind of food items such as soft animal tissues, detritus, hard invertebrate remnants and plant materials that can not be numerically or gravimetrically quantified (WINDELL, 1968 ; HYSLOP, 1980 ; JANSSENS DE BISTHOVEN *et al.*, 1990). The visual estimate of the stomach fullness was made using the scale of HYNES (1950), modified by WINDELL, 1968) and OLATUNDE (1978) (Table I).

TABLE I

Visual estimate of stomach fullness

Stomach replenishment	Points
Full (distended stomach)	100
3/4 full	75
1/2 full	50
1/4 full	25
trace	1-5
Empty	0

Mean stomach fullness index (MFI) with respect to capture time and food items categories was calculated using the following formula :

$$MFI = \Sigma Vc/Ne$$

$$VC = (Nv/Ne).100$$

where Vc is the estimated fullness of the stomach (points-method); Ne = total number of stomachs examined; Nv = total number of empty stomachs and VC = coefficient of empty stomachs (or the percentage of occurrence of fish with empty stomachs).

A one way analysis of variance has been used to compare different mean values of the stomach fullness indices (MFI) and prey items according to the time of the day.

RESULTS

General aspects of food and feeding habits

A total number of 689 fish ranging from 43 to 126 mm standard length (SL) were collected on 6 sampling days (Table II).

TABLE II

Numbers and size range of fish examined as collected from Kibuye and Gisenyi Baies

	(n)	size range (SL in mm)
Haplochromis graueri BOULENGER, 1914	159	48-126
Haplochromis nigroides PELLEGRIN, 1939	182	47-80
Haplochromis olivaceus SNOEKS, DE VOS, COENEN and THYS, 1990	184	43-90
Haplochromis kamiranzovu SNOEKS, COENEN and THYS, 1984	164	49-76

The food of these *Haplochromis* species consists of at least 21 different major prey items. As indicated by the gut content analysis, 3 major trophic groups were recognized, namely detritivorous (mud-feeder), microherbivorous or microphytophagous and insectivorous (Table III).

(1) *Mud-feeder :* this group is represented by *H. nigroides*. The gut content of this species consists almost entirely of microbenthic material, silts and detritus. The stomach of *H. nigroides* contains typically a mass of grey-black materials which after examination in suspension, revealed detritus and benthic organisms ranging from diatoms, protozoans, insect remains, ostracods and substantial amounts of grey-green mud which characterizes mud-feeder fishes.

(2) *Microphytophagous :* the group includes two species : *H. kamiranzovu* and *H. olivaceus*. Gut contents were dominated by substantial quantities of algae of benthic, epiphytic, epilithic and pelagic origin. The diet of *H. kamiranzovu* was mainly composed of the pelagic algae *Microcystis flos aquae* and a small quantity

of zooplankton especially copepods and cladocerans. The stomach of *H. olivaceus* contained significantly more periphyton, diatoms, epiphytic algae and a larger proportion of detritus. *Cladophora* sp. was frequently ingested by *H. olivaceus* in Gisenyi Bay while at Kibuye Bay the stomach content is dominated by *Callothrix*

TABLE III

Mean volume (MÆ) and percentage (%) in points-volume of various food items recorded in the stomach of 4 Haplochromis *species from lake Kivu*

	Species							
	H. olivaceus		*H. kamiranzovu*		*H. graueri*		*H. nigroides*	
Food items	MÆ	%	MÆ	%	MÆ	%	MÆ	%
Fish remains	0.05	0.08	0.09	0.13	—	—	0.03	6.61
Detritus	14.74	22.25	4.13	1.59	1.41	3.13	17.04	27.95
Sediments	4.64	7.00	0.77	1.08	1.99	4.41	0.33	0.54
Diatoms	4.72	7.12	0.26	0.37	0.15	0.33	1.17	1.92
Chironomids (larvae)	0.75	1.13	0.28	0.39	34.77	77.10	1.83	3.00
Nematodes (P)	0.34	0.51	0.08	0.11	—	—	0.13	0.21
Rotifera	0.04	0.06	0.02	0.03	—	—	0.14	0.23
Cladocera	0.03	0.05	3.36	4.79	0.63	1.40	5.73	9.4
Copepods	3.47	5.24	6.59	9.26	3.20	7.10	8.63	14.15
Eggs (unidentified)	0.99	1.49	0.16	0.22	1.08	2.39	1.69	2.77
Ostracods	0.07	0.11	0.09	0.13	0.26	0.58	0.13	0.21
Macrophyt remaons	0.43	0.65	0.38	0.53	0.55	1.22	0.82	1.34
Algae	33.64	50.78	54.01	80.07	0.03	0.07	9.20	15.09
Acanthocephala (P)	0.02	0.03	0.02	0.03	—	—	0.02	0.03
Acarina (P)	—	—	—	—	0.01	0.02	0.13	0.21
Diptera (pupae)	0.23	0.35	0.15	0.21	0.45	1.00	2.67	4.38
Plecoptera (nymphs)	—	—	—	—	—	—	0.49	0.80
Ephemeroptera (nymphs)	0.41	0.62	—	—	—	—	1.85	3.05
Insect remains	—	—	—	—	—	—	0.27	0.44
Hymenoptera	—	—	—	—	—	—	0.16	0.26
Unidentified animal remains	1.68	2.54	0.81	1.14	0.57	1.26	4.93	8.09

(—) represents differents item categories which are not recorded in the stomach.
(P) = intestinal parasites

epiphytica and *Schizothrix* sp. Considering the internal teeth composed of 4 to 9 rows, *H. olivaceus* shows « scraper » and « browser » characteristics.

(3) *Carnivorous : H. graueri* represents this group. It is a bottom feeder in which the main food is composed of dipteran larvae and pupae, especially chironomid larvae with variable quantities of other insects (terrestrial and aquatic), ephemeropterans, anisopterans and hemipterans. A very small quantity of copepods, cladocerans, detritus and sediments is also registered in the stomach of *H. graueri.*

Catch rates (Gisenyi Bay, February 12 and 17, 1987)

The catch rates showed 2 major peaks, when taking into account all 4 species studied together. The first peak appears almost invariably in the morning between 6.00 and 9.00 a.m., and the second in the afternoon between 3.00 and 6.00 p.m. (Fig 2). It is lower at noon and the lowest catch rate is recorded during the night. The mean number of fish captured during daytime appeared highly significantly different from the night catch rate ($P \leq 0.001$).

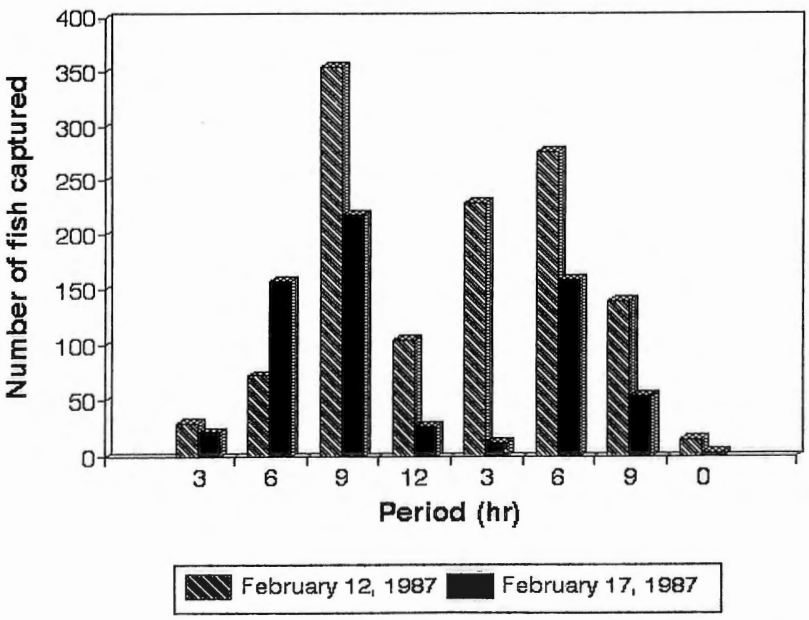

Fig. 2. — Catch-rates of *Haplochromis* species expressed as the total number of fish captured in Gisenyi Bay, February 12 and 17, 1987. (hr = hour)

Considering the catch rate for each species separately as represented in Fig. 3, the highest capture of *Haplochromis kamiranzovu* occurred in the afternoon from 3.00 to 9.00 p.m. This may indicate a vertical migration to search for food after

sunset. In the morning and during the day (between 9.00 a.m. and 9.00 p.m.), the rate of capture of *H. kamiranzovu* is significantly higher ($P \leq 0.05$) than of the others three species (*H. graueri, H. olivaceus* and *H. nigroides*). It can be hypothesized that *H. kamiranzovu* presents an active locomotory and feeding activity throughout the day till 9.00 p.m.

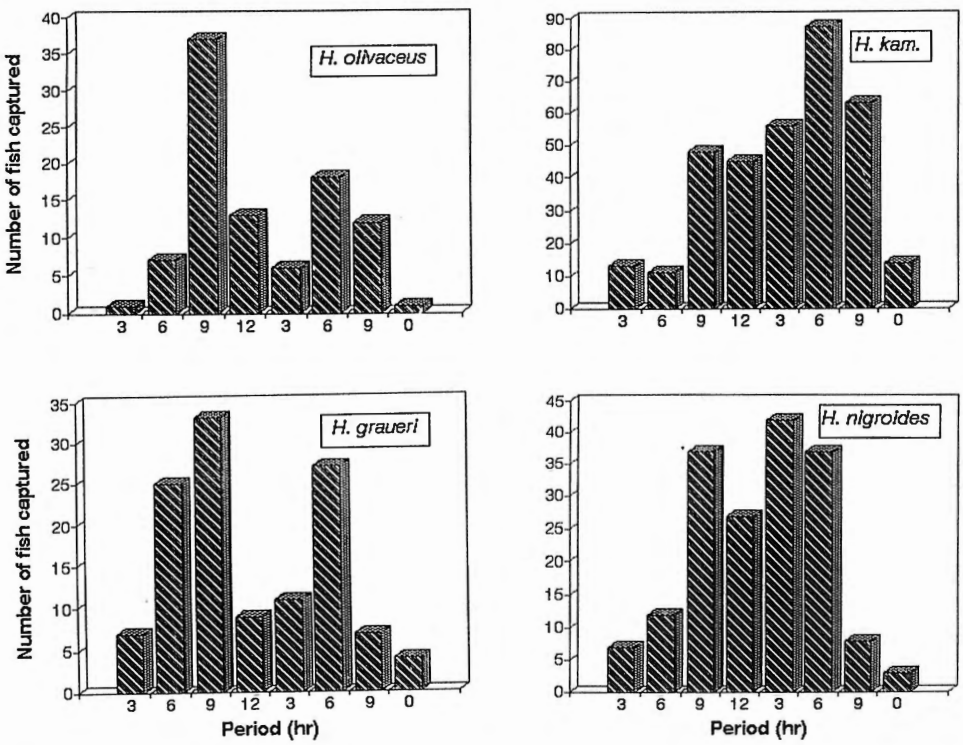

Fig. 3. — Catch-rates of four different *Haplochromis* species (*Haplochromis olivaceus, H. graueri, H. kamiranzovu* and *H. nigroides*), given as the total number of fishes captured during 3 cumulated 24-hour cycles. Abbreviation in the figure : *H. kamir.* = *H. kamiranzovu.*

Daily feeding rhythm

The analysis of the daily feeding activity pattern in the *Haplochromis* species of lake Kivu suggests that food uptake occurred mainly during daytime ($P \leq 0.01$) (Fig. 4, (a) and (b); Fig. 5 (a) and (b)). *H. graueri* and *H. olivaceus* indicate a bimodal feeding activity rhythm with the first peak situated in the morning between 6.00 and 12.00 a.m. and the second peak in the evening between 3.00 and 9.00 p.m.

In *H. graueri,* the means which characterize the major feeding peaks (9.00 a.m., MFI = 21.7 ± 7.3 and 9.00 p.m., MFI = 24.3 ± 13.8) show a highly significant difference with the means of all others periods ($P \leq 0.01$) (Fig. 4 (a)).

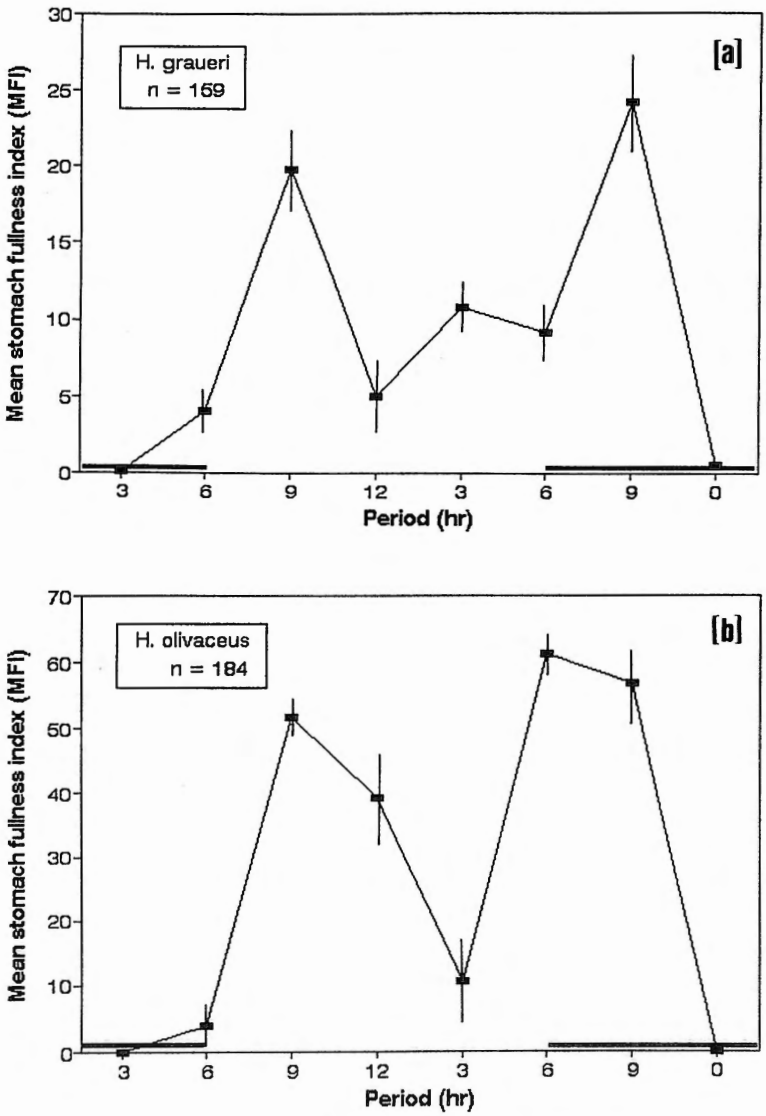

Fig. 4. — Daily feeding periodicity (in points-volume) of *H. graueri* (**a**), *H. olivaceus* (**b**). Fine curve (——) represents the mean points-volume of fullness index of the stomach at intervals of 3 hours of capture. Dotted solid bar (——) indicates hours of darkness. Vertical lines (|) represents the standard error on means (± SE).

The results obtained with *H. olivaceus* reveal like in *H. graueri* two daily feeding peaks, the first peak is observed in the morning at 9.00 a.m. (MFI = 51. ± 8.3) and the second at 6.00 p.m. (MFI = 61.1 ± 6.4) (Fig. 4 (b)) and continues till 9.00 p.m.

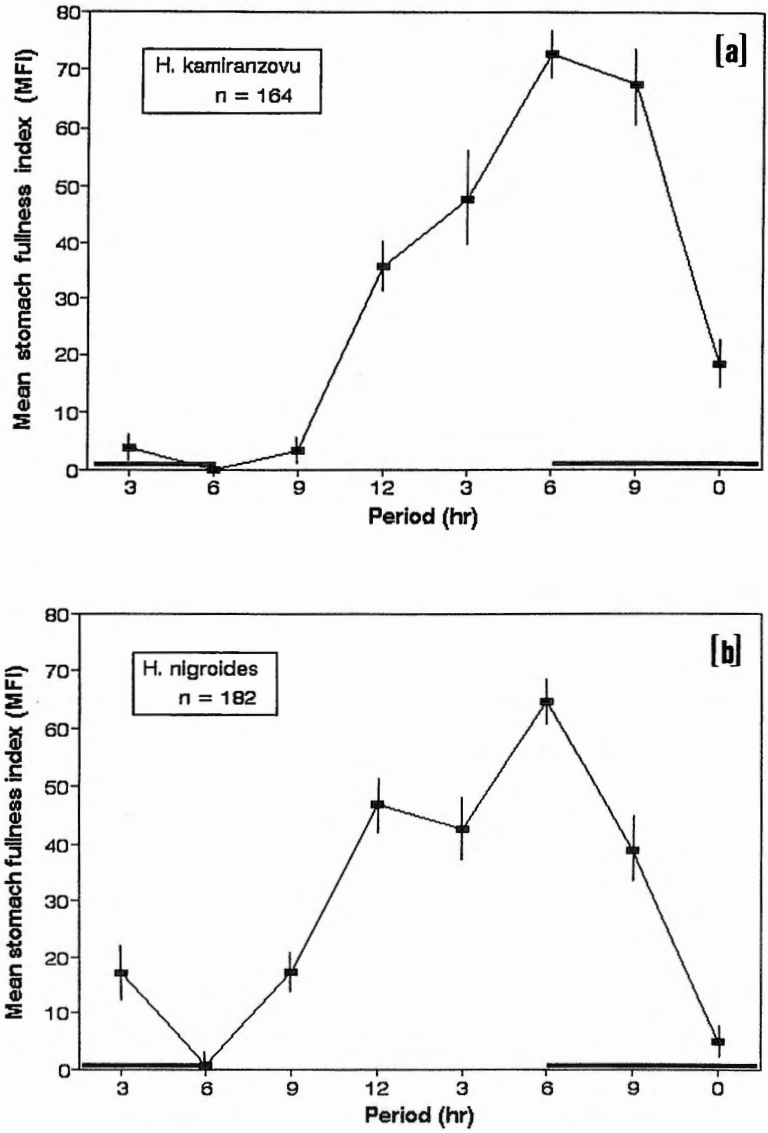

Fig. 5. — Daily feeding periodicity (in points-volume) of *H. kamiranzovu* (**a**) and *H. nigroides* (**b**). Fine curve (——) represents the mean points-volume of fullness index of the stomach at intervals of 3 hours of capture. Dotted solid bar (——) indicates hours of darkness. Vertical lines (|) represents the standard error on means (± SE).

Both mean volumes of feeding intensity are highly significantly different of the means of 3.00 and 6.00 a.m. ($P \leq 0.01$) and with 3.00 and 12.00 p.m. periods

($P \leq 0.001$) but not with the means of the feeding intensity of 9.00 p.m. (MFI = 56.7 ± 12.4, $P > 0.05$).

In both species, accelerated feeding activities started at about 6.00 a.m. and continued until 9.00 a.m. The stomach emptied slowly from that time onwards until around 12.00 a.m. in *H. graueri* and 3.00 p.m. in *H. olivaceus*. A low replenishment of the stomach is observed in most fish between 12.00 a.m. and 3.00 p.m.

In *H. graueri* for example, intermittent feeding occurred for a long period ranging between 12.00 a.m. to 6.00 p.m. during daytime. A high degree of variation of the degree of stomach fullness has been observed varying in some fish from empty stomachs up to full stomachs.

In contrast to this, *H. kamiranzovu* and *H. nigroides* show a single peak of feeding activity situated at 6.00 p.m. Active feeding started at about 6.00 a.m. and stomachs reach their maximum fullness at around 3.00 to 6.00 p.m. Thereafter, the stomachs remained nearly full until about 9.00 p.m. ($P \leq 0.05$) in *H. kamiranzovu,* but decreased rapidly in *H. nigroides* (Fig. 5 (a) and (b)).

Coefficient of vacuity (CV)

The highest percentages of empty stomachs were observed during the night-time while lower percentages were seen during the day ($P \leq 0.01$). The variation of the average vacuity coefficient in the various *Haplochromis* species studied here seems not only to be related with the dial feeding rhythm but also with the food items ingested (Table IV). It indicates that these *Haplochromis* species do not feed during the night. It can also be postulated that the highest capacity of stomach and intestinal storage must occur in daytime, while *Haplochromis* are inactive at night (THYS VAN DEN AUDENAERDE, 1986 and DE VOS *et al.*, 1987).

TABLE IV

Variations of coefficient of vacuity (CV) of 4 Haplochromis *species calculated as the percentage of occurence of empty stomachs over a 24-hour cycle in lake Kivu*

Hour (hr)	3 a.m.	6 a.m.	9 a.m.	12 a.m.	3 p.m.	6 p.m.	9 p.m.	0 p.m.
H. kamiranzovu	92.3	100.0	35.71	7.6	4.1	5.8	10.8	57.1
H. nigroides	40.0	80.0	40.9	16.3	14.3	14.8	37.5	66.7
H. graueri	85.7	84.0	39.4	64.9	58.6	59.3	42.9	75.0
H. olivaceus	100.0	71.4	18.9	7.7	66.7	5.7	3.3	100.0

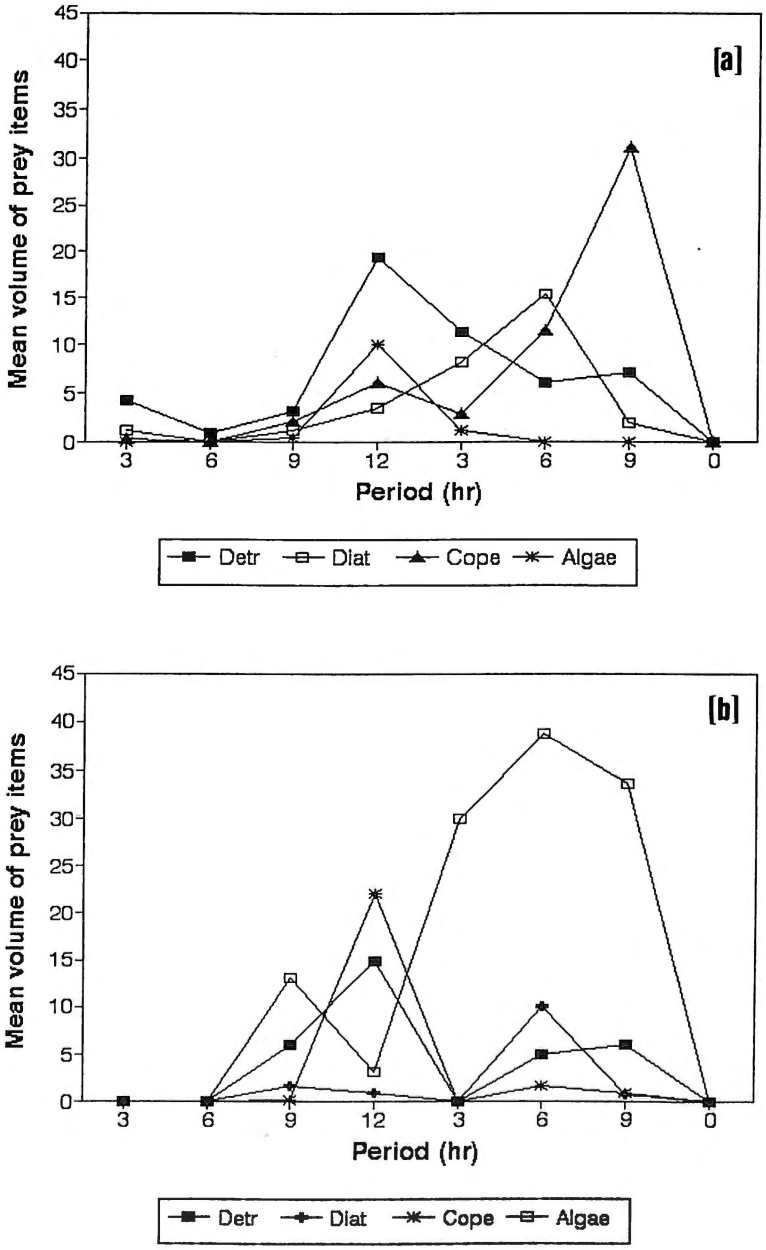

Fig. 6. — Daily dietary changes of food ingested by *H. nigroides* (**a**), *H. olivaceus* (**b**). Detr = detritus ; Diat = diatoms ; Cope = copepods ; Chir = chironomids larvae ; Clad = cladocerans

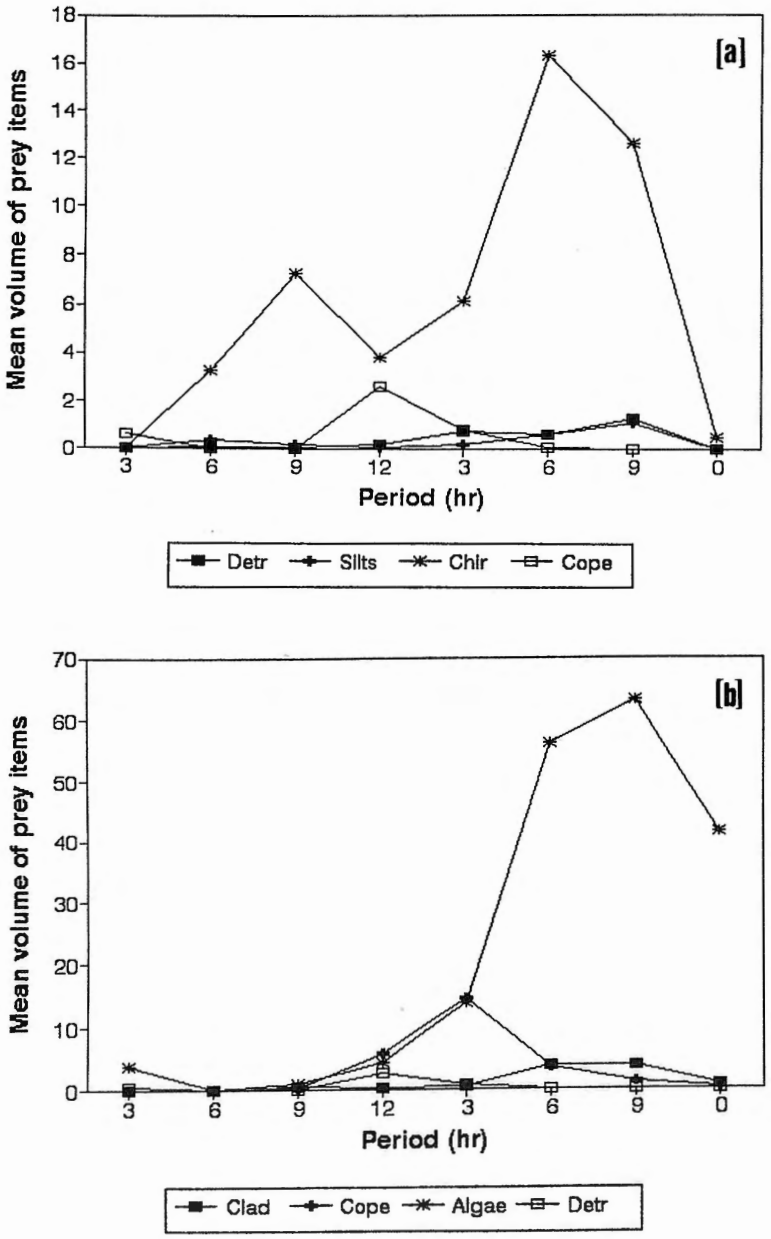

Fig. 7. — Daily dietary changes of food ingested by *H. graueri* (**a**) and *H. kamiranzovu* (**b**) (see legends in Fig. 6).

Changes in kinds of food eaten during 24-hours period

All *Haplochromis* species studied presently showed changes in the prey items taken according to feeding periods. Prey items eaten during the day are significantly different from prey items recorded in the stomachs of fish captured during the night ($P \leq 0.001$). This is detected in most of the cases by a high number of empty stomachs and low mean volume of prey eaten during the night and in some cases, by a total absence of feeding activity throughout the night (Table IV ; Fig. 6 (a) and (b) ; Fig. 7 (a) and (b)).

H. nigroides presents a pretrophic feeding activity before sunrise. Three prey categories are apparently present in the stomach at 3.00 a.m. ; they consist of algae, copepods and detritus (Fig. 6 (a)). But the ingestion of these preys decreases significantly in the first half of the day (6.00 a.m.), and then increases significantly at 12.00 a.m. until 3.00 to 9.00 p.m.. On the other side, predation on copepods and algae, mainly benthic algae *Cladophora* sp., goes on increasingly from 9.00 a.m. to 6.00 and 9.00 p.m. respectively (Fig. 6 (a)).

In contrast, for *H. olivaceus* (which has the same microphytophagous diet as *H. kamiranzovu*), the regime is composed of various kind of prey items in the morning till 12.00 a.m. (copepods, organic matter or detritus, algae). In the second half of the day, *H. olivaceus* feeds almost exclusively on multicellular algae *Cladophora* sp. Diatoms and detritus occured also in the stomach, but in lower proportion than algae (Fig. 6 (b)). *H. graueri* does not show a major variation in the nature of food ingested during whole the day. Chironomid larvae, especially *Chironomus* type *plumosus* and *Tanipus* sp. are the most frequently ingested species. The mean points-volume of other prey items captured by this species presents less variation (Fig. 7 (a)). Despite the evidence for the regular intake of chironomid larvae throughout the day, a decrease in mean volume of chironomid larvae is observed at about 12.00 a.m. Copepods are apparently an important prey item captured at that time (Fig. 7 (a)).

For *H. kamiranzovu,* which feeds almost throughout the day, the main prey eaten remains colonial unicellular algae *Microcystis flos aquae* and copepods. A small quantity of organic matter was found frequently between 12.00 a.m. to 6.00 p.m. indicating that this species feeds near or at the bottom during this period. While copepods show the highest peak at 3.00 p.m., a small proportion of cladocerans in the stomach of *H. kamiranzovu* is observed between 6.00 and 9.00 p.m.. (Fig. 7 (b)).

DISCUSSION AND CONCLUSION

The present study shows that these four *Haplochromis* species from lake Kivu feed during the daytime. The peak of the stomach fullness index appears almost invariably 3 hours after the start of active foraging, detected by a rapid rise in number of fish captured. This is most clearly represented in fishes showing two distinct feeding peaks such as *H. graueri* and *H. olivaceus* (Fig 4 (a) and (b)).

However, in the case of *H. kamiranzovu* and *H. nigroides,* fresh food was almost always present in the stomach at all times of the day, which would suggest continuous and active feeding (ALBERTINI-BERHAUT, 1974 ; ROBB, 1981). (Fig. 5 (a) and (b)).

Catch rates in *Haplochromis* may well be a measure of pre-feeding activity which is reflected by a rapid increase of the average number of fish captured and the fullness index data in this study support such interpretation (Fig. 2). Low catch rates observed in *Haplochromis* from lake Kivu during daylight (between 9.00 a.m. and 3.00 p.m. on February 17, 1987) may be explained by either a low locomotory activity or a possible avoidance behaviour due to the visibility of the fishing nets (RYAN, 1984). It is known that most *Haplochromis* in lake Kivu do not swim over long distances, especially territorial adult males do not migrate. It can be assumed that the main reason for swimming is the search for food (THYS VAN DEN AUDENAERDE, 1986), for the reproduction or the attraction of females. According to THYS VAN DEN AUDENAERDE (1986), *Haplochromis* species are more or less asleep at night. During the night they remain almost immobile on/or near the bottom (THYS VAN DEN AUDENAERDE, 1986, DE VOS *et al.,* 1987). This assertion corresponds to our findings reflected by very low catch rates overnight (Fig. 2) and high percentages of empty stomachs (Table IV). *Haplochromis* species become active at sunrise or shortly after the first daylight (THYS VAN DEN AUDENAERDE, 1986).

This situation is clearly reflected by higher percentages of full stomachs ranging from 50 to 100 in most species between 6.00 and 9.00 a.m. But *H. kamiranzovu* which shows a single peak of diurnal feeding activity, seems also to be active overnight : a part of or the whole population seems to migrate vertically toward the surface before dawn (DE VOS *et al.,* 1987). This situation is clearly demonstrated in this study by the stomach fullness index recorded at 9.00 a.m. till 9.00 p.m., and a relatively high number of fish captured between 3.00, 6.00 and 9.00 p.m. as represented in fig. 2 b.

It can also be suggested that the *Haplochromis* species which were studied prey on various kinds of food items according to the accessibility of preys and the preference and feeding habits od the predators themselves (KEAST and WELSH, 1968 ; NEVEU, 1981a ; SCRIMGEOUR, 1986). Studying feeding periodicity in *Cheimarrichthys forsteri* and *Gobiomorphus hubsi* in New-Zealand River, SCRIMGEOUR and WINTERBOURN (1987) reached the conclusion that by feeding predominantly at different times of the night, the competition between the two species for food, should it be in short supply, was reduced. The different feeding periods of the various fish species of lake Kivu probably influence the selection of the food obtained.

Differences in feeding strategy ensure that all potential food organisms are cropped by at least one fish species during a determined period. According to MACPHERSON (1985), differences in feeding activity may also be due to internal rhythms independent of food availability. Many factor, internal as well as external, which have negative or positive effects on food and feeding habits can be evoked. Feeding activities are influenced by physical (temperature, light intensity, turbidity, eutrophication...) and chemical factors ($C0_2$, pH, 0_2 ...) as well as by biological and

physiological aspects (migration of plankton, digestion, evacuation rates, etc.) and many others (NEVEU, 1981a). DE VOS *et al.*, 1987 studied the vertical migration of some *Haplochromis* in lake Kivu and suggested that the nocturnal vertical migration in *Haplochromis* can be considered as a feeding migration. A similar phenomenon has been observed in many other *Haplochromis* species from lake Victoria (WITTE, 1984 ; GOLDSCHMIDT *et al.*, 1990). In crater lakes in West Cameron, it was also observed that *Haplochromis* species migrate according to vertical movements of larvae and nymphs of *Chaoborus* (GREEN *et al.*, 1973). DE VOS *et al.* (1987) concluded that the migration of some *Haplochromis* species from lake Kivu should be explained by the migration and movement of nematocerans. It has also been observed that chironomid and trichopteran pupae generally move vertically through the water column prior to emerging as adults mostly at night although some of them do so in the forenoon and afternoon. This migration explains, at least partially, the importance and a proportional value of chironomids and trichopterans in the stomach of insect larvae eaters, such as *Haplochromis graueri,* throughout the day and sometimes also later at night.

The variation of the average vacuity coefficient registered in the different *Haplochromis* species studied here seems to be related with the dial feeding rhythm pattern and with the categories of prey items ingested. The high percentage of empty stomachs, the low degree of capture and the low index of stomach fullness during the night indicate that feeding activity declines significantly throughout the night.

We observed that the coefficient of vacuity is higher in carnivorous Haplochromis species such as *H. graueri* than in omnivorous fish like *H. gracilior* (in preparation), in detritivorous *(H. nigroides,)* and in algae eaters (*H. olivaceus* and *H. kamiranzovu)*. This may also indicate that carnivorous fishes have a relatively high digestion rate and feed less actively than detritus and algae eaters. An experimental study in the laboratory with *Haplochromis* spp. from lake Kivu, fed with dry food, coloured with chromoxide, showed that the evacuation time necessary can be estimated to range from 2 to 3 hours (pers. observ.).

Dietary changes over a 24-hour cycle show that some prey items are more accessible at specific hours of the day (Fig. 6 (a),(b) ; Fig. 7 (a) and (b)). The relation between the uptake of different prey types and feeding time over the 24-hour periods reflects that each Haplochromis species in lake Kivu has developed an appropriate strategy which enables it to crop potential food items at these periods of the day. This leads probably to a further reduction or in some cases even to a complete absence of interspecific food competition (KEAST and WELSH, 1968 ; NEVEU, 1981b ; LECOMPTE-FININGER, 1983 ; SCRIMGEOUR and WINTERBOURN, 1987 ; DELBEEK and WILLIAMS, 1988).

Differences in feeding time can serve to reduce interspecific contact and competition, at least in the cases of versatile and generalized feeders (KEAST and WELSH, 1968), such as *Haplochromis nigroides, H. olivaceus* and *H. graueri.* Recent findings (ULYEL *et al.*, 1990) showed that *Haplochromis* species from lake Kivu do not compete for major food items of their regimes. Each species occupies in the lake a well defined ecological habitat, sufficiently distinct from others so that competition for

important prey is avoided (ULYEL *et al.* 1990). In conclusion, it can be considered that spatial and temporal exploitation and the use of different resources are an important factor in organizing fish communities. Because fishes, in the presence of potential competitors under natural conditions, alter their behaviour in order to reduce the utilisation of similar resources (WERNER, 1984).

Sometimes, modifications of anatomical structures may occur, virtually independent of size or age (WITTE *et al,* 1990). The configurations of such anatomical structures may depend also on the ontogenetic stage (such as age and size) at which the environmental switch occurs (HOOGERHOUD, 1987; WITTE *et al.,* 1990). Morphologically, most of the trophic groups are recognizable by a particular facies (BAREL, 1983). A facies and its constituent structures are related to the way food is processed rather than to the food type itself. In *Haplochromis* species from lake Kivu, this assertion may be illustrated in some trophic groups, such as in carnivorous species *H. vittatus* and *H. graueri* (LT $\geq$ 75 mm), by several characterstics like canine-like teeth on the outer-rows on both upper and lower jaws and by relatively protrusible and strong jaws. According to our preliminary studies on dentition structures of *Haplochromis adolphifrederici,* the relationships between ecology and morphology with regard to diet composition can be illustrated by submolariform teeth covering the entero-central part of the lower pharyngeal bones in juveniles and subadult specimens, while they are molariform in adults. Such a degree of development of molariform teeth, as demonstrated in *Astatoreochromis alluadi* from lake Victoria (WITTE *et al.,* 1990), is probably correlated with the relative abundance of molluscs in the diet of the adult of *H. adolphifrederici.* On the other hand, we can hypothesize in the present study that the efficiency of *H. olivaceus* to scrape epilithic algae increases with the numerous rows (4 to 9) of internal tricuspids teeth on both upper and lower jaws.

Since *Haplochromis* from lake Kivu feed on different prey types, different sizes of prey (in preparation) and at different times of the day, the present observations support the view that these species are ecologically isolated. For example, in the presence of a variety of organisms, differences in diet would result from predators following the strategy to which they are best adapted and, in this way, if food was a limiting factor, interspecific competition could be avoided or reduced (ROBB, 1981), and most of the species would become versatile. Further, species apparently specialized for certain food sources, such as *H. kamiranzovu* in lake Kivu, may switch food preferences when other foods become superabundant (WITTE, thèse de doctorat, 1983).

ULYEL *et al.,* (1990) suggested that rare cases in which competition for food occurs among *Haplochromis* species of lake Kivu concerns very few prey items of minor importance. This suggestion supports the hypothesis of a partial overlap of some *Haplochromis* species in the use of relatively abundant prey items and the absence of marked food competition which can be considered as a consequence of adaptive radiation resulting in distinct feeding habits of the different species and/or the consequence of fixed adaptations due to a competition which occured in the past (ULYEL *et al.,* 1990). As claimed by GOLDSCHMIDT *et al.* (1990), the presence of distinct segregation patterns in the absence of interactive competition does not

prove that competition never existed. Another possibility, as suggested by ROBB (1981), is that differences in diet may result from differences in feeding behaviour itself. Analysing ecological segregation in zooplanctivorous *Haplochromis* spp. from lake Victoria, GOLDSCHMIDT *et al.* (1990), stated that these species are ecologically segregated and therefore possibly sufficiently isolated to coexist.

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A STUDY OF FEATHERPRINTS BY SCANNING ELECTRON MICROSCOPY

by

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SUMMARY

The surface of the rachis, rami and rachidial barbules of feathers of 109 species, belonging to 56 families and 17 orders, is investigated by means of the scanning electron microscope. Photographic illustrations, dealing with 18 species, demonstrating the wide diversity of featherprints, including micropapillae, fibrillary striations, pits and cell boundaries are presented.

Key Words : feather surface — featherprints — ultrastructure.

INTRODUCTION

Faced with identification problems when only a few feathers or parts of feathers are available, the ultrastructure of the feather surface was investigated.

On the rachis, the rami and the rachidial barbules of all investigated species a great diversity of ultrastructures was discovered. We call them featherprints. It became evident that these structures are interesting clues for purposes of identification.

The great diversity of structures found created first of all the need for a detailed enumeration and illustration with hypotheses about their possible function.

MATERIAL AND METHODS

Feathers were obtained from live birds, recently dead specimens and from museum collections. Whenever possible feathers from both sexes and also from juveniles and adults were collected.

Feathers from 14 different parts of the skin were used for intraspecific studies. These feathers include : tailfeathers, upper and under tail-coverts, primaries, upper

and under wing-coverts, feathers of the rump, the back, the nape, the crown, the throat, the breast, the belly and the flank.

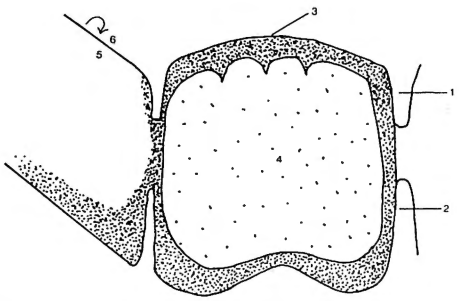


Fig. 1. — Cross section through a feather with obverso-lateral surface of the rachis (1), reverso-lateral surface of the rachis (2), obverse surface of the rachis (3), transverse section of the rachis (4), proximal surface of a ramus (5), distal surface of a ramus (6).

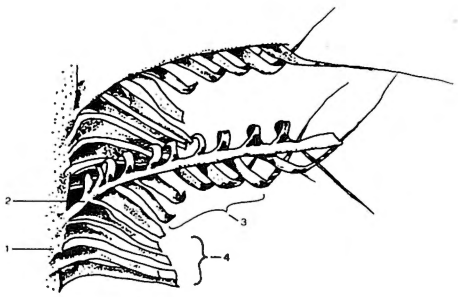


Fig. 2. — Position of the rachidial barbules with rachis (1), barb (2), barbules (3), rachidial barbules (4) (LUCAS and STETTENHEIM, 1972).

Nine different sites of a feather were examined with magnifications up to 20,000 × : I = reverso-lateral surface of the rachis below the rami ; II = reverso-lateral surface of the rachis between the rami ; III = proximal surface of a ramus below the barbules ; IV = distal surface of a ramus below the barbules ; V = reverse surface of the base of the rachidial barbules ; VI = obverso-lateral surface of the rachis between the rami ; VII = proximal surface of a ramus above the barbules ; VIII = distal surface of a ramus above the barbules ; IX = obverse surface of the rachis.

The nomenclature of feather parts corresponds to LUCAS and STETTENHEIM (1972) and the terminology of feather orientation to DYCK (1971a) (cfr. Fig. 1 and 2).

Only the ninth primary was used for interspecific comparisons because the featherprints turned out to be intraspecifically stable (PERREMANS, 1990).

A scanning electron microscope (SEM 515, Philips) was used, allowing continuous magnifications from 20 × up to 160,000 × at a resolution of 5 nm and a focal depth of 20 µm.

Prior to investigation the feathers were washed and degreased. Small pieces of rachis (± 1 cm) with cut-off barbs were used. The relevant parts were attached to specimen stubs by means of two-sided cellotape and coated with gold (30 nm) in a « High Vacuum Gold Sputter-Coater » (Balzers Union).

Feathers of 109 species belonging to 56 families and 17 orders were examined.

RESULTS

A great diversity of feather surface structures and markings was observed, appearing in most species in a unique combination.

Micropapillae were present in 45 species on the nine examined feather sites. They appeared in various shapes : short as in the Long-tailed Cormorant (*Phalacrocorax africanus* (GMELIN, 1789)) (Pl. 1, A), long as in the Shoebill (*Balaeniceps rex* GOULD, 1850)(Pl. 1, B) ; in various densities : a high density as in the Pink-backed Pelican (*Pelecanus rufescens* GMELIN, 1789) (Pl. 1, C), a low density as in the Grey Crowned Crane (*Balearica regulorum* (BENNETT, 1834)) (Pl. 1, D) and in various configurations as in the Swift (*Apus apus* (LINNAEUS, 1758)) (Pl. 1, E).

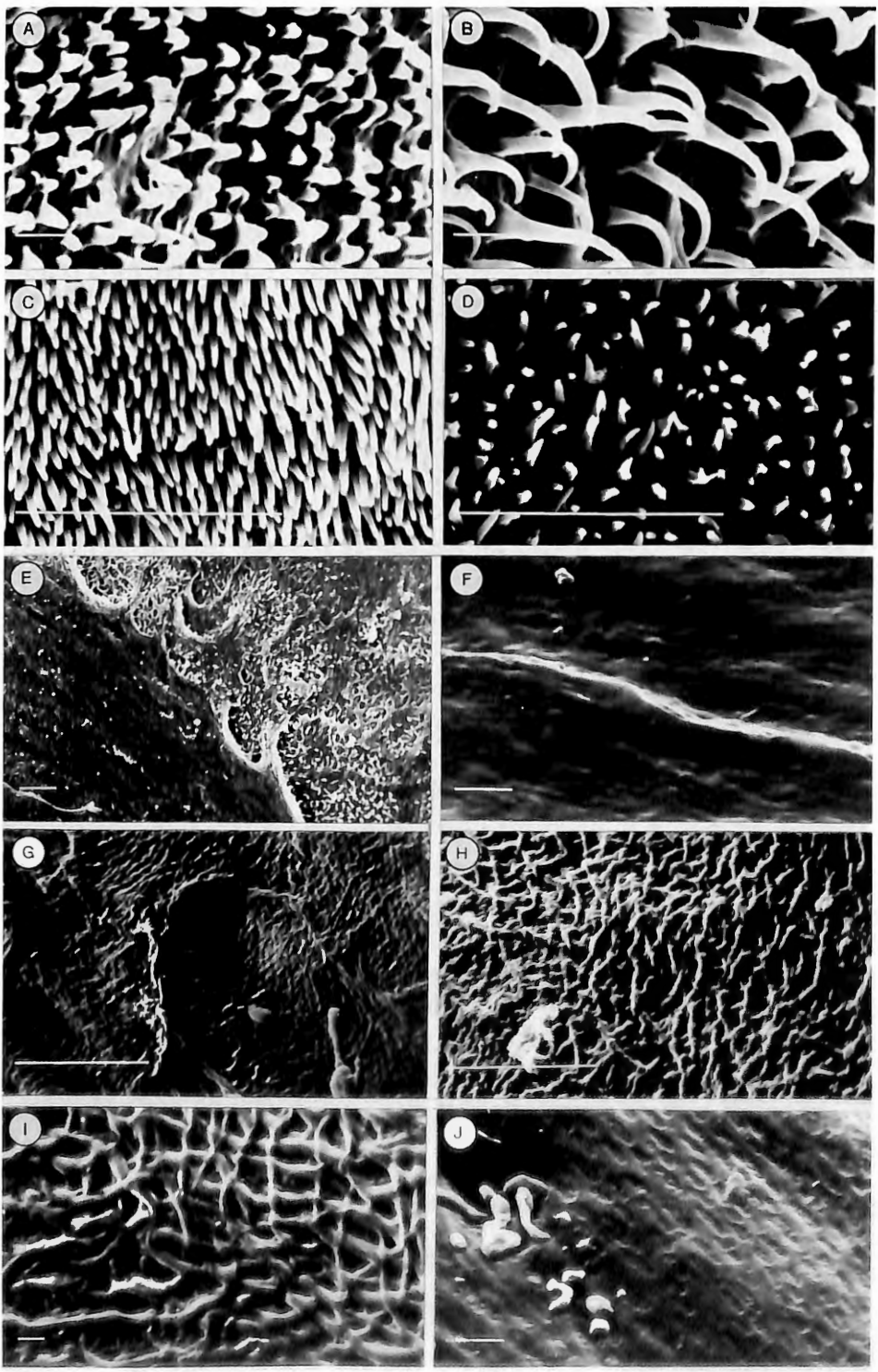
The surface of the rachis, the rami and the rachidial barbules varied also from relatively smooth to very roughly frayed as shown on Pl. 1, F to I : relatively smooth on the obverse surface of the rachis in the Great Tit (*Parus major* LINNAEUS, 1758) (F) ; finely frayed on the reverso-lateral surface of the rachis below the rami in the Robin (*Erithacus rubecula* (LINNAEUS, 1758)) (G) ; roughly frayed on the proximal surface of a ramus below the barbules in the Puffin (*Fratercula arctica* (LINNAEUS, 1758)) (H) ; very roughly frayed on the reverso-lateral surface of the rachis between the rami in the Moorhen (*Gallinula chloropus* (LINNAEUS, 1758)) (I). The surface could also be roughly frayed in the presence of a few micropapillae.

Another type of texture of the rachis included pits. Very small pits were found on the obverse surface of the rachis in the Woodpigeon (*Columba palumbus* LINNAEUS, 1758) (Pl. 1, J), deep pits on the same surface in the Puffin (*Fratercula arctica*) (Pl. 2, A), deep pits containing a core also on this surface in the Lesser Flamingo (*Phoenicopterus minor* GEOFFROY SAINT-HILAIRE, 1798) (Pl. 2, B), great deep pits on the reverso-lateral surface of the rachis below the rami in the Green Woodpecker (*Picus viridis* LINNAEUS, 1758) (Pl. 2, C) and a honey comb structure on the obverse surface of the rachis in the Vulturine Fish Eagle (*Gypohierax angolensis* (GMELIN, 1788)) (Pl. 2, D).

On all sites the surface showed cell boundaries. Five different types were distinguished (Pl. 2, E to I). They were found as fine, deep laying lines (type 1) on the proximal surface of a ramus below the barbules in the Gannet (*Sula bassana* (LINNAEUS, 1758)) (E), as fine, rising lines (type 2) on the distal surface of a ramus below the barbules in the Green-backed Heron (*Butorides striatus* (LINNAEUS, 1758)) (F), as thick, rising lines (type 3) on the reverso-lateral surface of the rachis below the rami in the Snipe (*Gallinago gallinago* (LINNAEUS, 1758)) (G), as incomplete cell boundaries (type 4) on the reverso-lateral surface of the rachis in the Collared Dove (*Streptopelia decaocto* (FRIVALDSZKY, 1838)) (H) and as very thick lines (type 5) on the reverso-lateral surface of the rachis below the rami in the Mallard (*Anas platyrhynchos* LINNAEUS, 1758) (I). The cell boundaries supposedly correspond to the cell borders of the original surface cells.

Cell walls could be protruding or flat. Protruding cell walls (Pl. 2, J) were found on the obverse surface of the rachis in the Gannet (*Sula bassana*). In a lot of

PLATE 1



specimens an oval depression, possibly corresponding to the original position of the nucleus of the cell, was found.

The structures on the lateral surface of the rachis and on the proximal and distal surface of the rami were in most cases more pronounced on the reverse part of the feather, although in some species they appeared with the same intensity on the obverse part. Usually the same structure was found at the proximal and distal surface of a ramus — close to the attachment of the ramus with the rachis — as on the corresponding lateral rachis surface. These structures became nearly always less and less distinct away from the attachment although in some species the rami showed no fading of the structures along the remaining barb piece. In still other species the structures on the rami surfaces differed from those observed on the lateral surface of the rachis.

The reverse surface of the base of the rachidial barbules may be frayed or may carry micropapillae. Such micropapillae have up to now only been found in the Rock Dove (feral form, *Columba livia* GMELIN, 1789), the Woodpigeon (*Columba palumbus*) and the Collared Dove (*Streptopelia decaocto*).

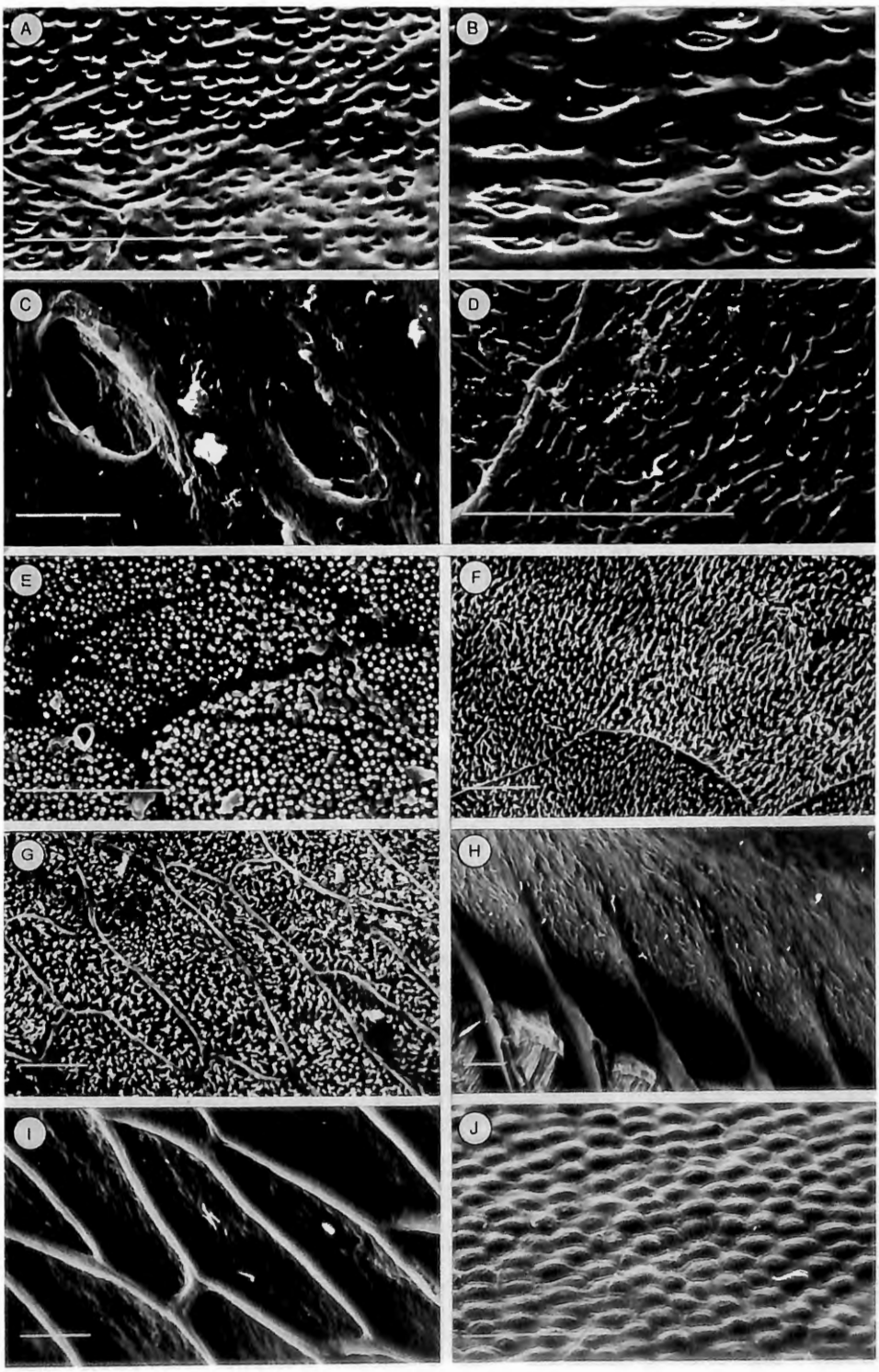
DISCUSSION

Rachis and rami of the developing feather are composed of two layers of cells : a thick inner core of spongy polygonal medulla cells and a thinner outer layer of compact amorphous cortex cells (STRONG, 1902, OLSON, 1970, LUCAS and STETTENHEIM, 1972, BLEIWEISS, 1987).

PLATE 1

Micropapillae in various shapes : A. Long-tailed Cormorant (*Phalacrocorax africanus*) : short micropapillae on the reverso-lateral surface of the rachis below the rami (bar = 1 µm). B. Shoebill (*Balaeniceps rex*) : long micropapillae on the reverso-lateral surface of the rachis between the rami (bar = 1 µm). **Micropapillae in various densities :** C. Pink-backed Pelican (*Pelecanus rufescens*) : micropapillae in a high density on the reverso-lateral surface of the rachis between the rami (bar = 10 µm). D. Grey Crowned Crane (*Balearica regulorum*) : micropapillae in a low density on the proximal surface of a ramus below the barbules (bar = 10 µm). **Micropapillae in various configurations :** E. Swift (*Apus apus*) : the reverso-lateral surface of the rachis below the rami (bar = 10 µm). **Frayed : relatively smooth :** F. Great Tit (*Parus major*) : the obverse surface of the rachis (bar = 1 µm). **Frayed : finely frayed :** G. Robin (*Erithacus rubecula*) : the reverso-lateral surface of the rachis below the rami (bar = 10 µm). **Frayed : roughly frayed :** H. Puffin (*Fratercula arctica*) : the proximal surface of a ramus below the barbules (bar = 10 µm). **Frayed : very roughly frayed :** I. Moorhen (*Gallinula chloropus*) : the reverso-lateral surface of the rachis between the rami (bar = 1 µm). **Pitted : very small pits :** J. Woodpigeon (*Columba palumbus*) : the obverse surface of the rachis (bar = 1 µm).

PLATE 2



A very thin layer, called the epitrichium, covering the cortex of the rami was mentioned by HAECKER (1890). Similarly DYCK (1971a, 1979) described a thin, dense epicuticle covering the surface of the rami and barbules. The cortex of the rachis and rami is further subdivided in two layers (CLÉMENT, 1876, AUBER and APPLEYARD, 1951, RUTSCHKE, 1966, DYCK, 1971a,b) :

1. The outer layer or surface layer consists of flattened polygonal cells. These cells form a pattern similar to some of the cuticular scale patterns found on mammalian hairs (AUBER and APPLEYARD, 1951, 1955). OLSON (1970) could not distinguish cells in the surface layer of any of his specimens under a light microscope. DYCK (1971a, 1973) concluded that it is very unlikely that acellular barb cortexes exist based on observations with the electron microscope.

2. The inner layer or cortex proper (AUBER and APPLEYARD, 1951) consists of cells that are longer and less flattened than the surface layer cells. They are spindle-shaped and show some similarity to the cortical cells in mammalian hair.

As long ago as in 1918 GLADSTONE observed a network of polygons on the tegmen of a primary from the Pink-footed Goose (*Anser brachyrhynchus*). The magnifications he used were too small to distinguish fine details. AUBER and APPLEYARD (1951) confirmed these observations and found a network of polygons arranged with their long axes parallel to the length of the barb, on the ventral surface of the barbs of Guinea fowl (*Numida sp.*), « Silky » fowl (*Gallus sp.*) and Green Honeycreeper (*Chlorophanes spiza*). They also mentioned the appearance of coarse striations and fibrillary structures. They suggested these patterns were formed by the superimposition of the fibrillary striation within the surface cells upon the parallel fibrillary striations of the cortex proper. DYCK (1973) concluded that the surface cells show structures that are formed by the cells themselves. Based on our observations we agree with his conclusions.

PLATE 2

Pitted : deep pits : A. Puffin (*Fratercula arctica*) : the obverse surface of the rachis (bar = 1 μm). **Pitted : deep pits containing a core :** B. Lesser Flamingo (*Phoenicopterus minor*) : the obverse surface of the rachis (bar = 1 μm). **Pitted : great deep pits :** C. Green Woodpecker (*Picus viridis*) : the reverso-lateral surface of the rachis below the rami (bar = 1 μm). **Pitted : honey comb structure :** D. Vulturine Fish Eagle (*Gypohierax angolensis*) : the obverse surface of the rachis (bar = 10 μm). **Type of cell boundary : type 1 = fine, deep laying lines :** E. Gannet (*Sula bassana*) : the reverso-lateral surface of the rachis below the rami (bar = 10 μm). **Type of cell boundary : type 2 = fine, rising lines :** F. Green-backed Heron (*Butorides striatus*) : the distal surface of a ramus below the barbules (bar = 10 μm). **Type of cell boundary : type 3 = thick, rising lines :** G. Snipe (*Gallinago gallinago*) : the reverso-lateral surface of the rachis below the rami (bar = 10 μm). **Type of cell boundary : type 4 = incomplete cell boundaries :** H. Collared Dove (*Streptopelia decaocto*) : the reverso-lateral surface of the rachis (bar = 10 μm). **Type of cell boundary : type 5 = very thick lines :** I. Mallard (*Anas platyrhynchos*) : the reverso-lateral surface of the rachis below the rami (bar = 10 μm). **Cell surface : protruding cell walls :** J. Gannet (*Sula bassana*) : the obverse surface of the rachis (bar = 100 μm).

The longitudinal fibrillary striation probably can be explained by the longitudinal orientation of the fibrillary proteins constituting the cell interior of the surface layer cells. The question remains how micropapillae, pits and honey comb structures are formed.

For the villi-like outgrowths of the rachis, the rami and the rachidial barbules the term micropapillae has been adopted from LYSTER (1985, unpublished report). The reason for this is that the term villi is in use already for the outgrowths on the bases of the basalmost downy barbules (CHANDLER, 1916, BROM, 1986, 1990). These basal outgrowths are considered not to be homologous to ours.

The function of these surface structures is still unclear. Probably they do not play a role in the modification of feather gloss because they were mainly found on the reverse parts of the feather. They seem too small for insulation purposes. Since they were found on the primaries and tail feathers as well as on the body contour feathers no cooperation in the flight function is supposed. A possible function (DYCK, pers. comm.) could be that they contribute to the creation of friction between different feathers.

Further analysis will give more information on the exact value of this set of characters for determination purposes and for avian taxonomy.

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CORRELATION BETWEEN THE WING LENGTH OF LIVING BIRDS AND MEASUREMENTS OF THEIR BONES

by

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SUMMARY

An indirect method useful in identifying fossil and recent bird bone remains is described. In birds belonging to one group (genus, family) of similar body plan, various measurements of the bones are correlated with wing length. The latter parameter may be easily found in ornithological literature. The minimal and maximal measurements of the wing length compared with the minimal and maximal measurements of a bone, show linear arrangement in diagrams. It enables us to estimate the wing length on the basis of the size of a bone which may be helpful in determining the taxonomic position of the bone — even if the species is not represented in osteological collections.

Key words : bird, wing length, bone sizes, correlation, identification.

INTRODUCTION

Dimensions of bones play an important role in identifying fossil and contemporary bird remains. The complete data on skeletal measurement are available in literature only for a very limited number of recent bird species. Direct comparison with well-determined skeletal specimens is not always possible. There are more than 100 osteological collections in the world. However, specimens of rare birds are either well-dispersed or missing altogether (WOOD and SCHNELL, 1986). As a consequence, the comparative material for determination is sometimes hardly available. That is why it is often necessary to look for indirect methods. The purpose of this paper is to determine to what degree and in which cases the size of a bone fragment is connected with wing length (the latter parameter is commonly cited in ornithological literature). In other words, whether the size of a bone fragment can indicate the wing length which, in turn, may support the identification of the species.

MATERIAL AND METHODS

At first wing lengths of individual birds were compared with their bone measurements (the width of the distal articular part of the humerus and tibiotarsus) in the series of skeletal specimens stored at the Institute of Systematics and Evolution of Animals, Polish Academy of Sciences (ISEA). The wing length was measured before preparation from the carpal joint to the tip of the longest primary. The specimens represented two phylogenetically distant genera : *Larus* and *Corvus*. The results are shown in the diagrams (Fig. 1 A, B and 2 A, B). For each, the reduced major axis and the correlation coefficient were calculated (r between 0.93 and 0.97).

Afterwards, the same 4 species of the genus *Corvus* from Fig. 2 A and B and the same parameters were studied in a different way. The minimal and maximal measurements of the wing lengths were plotted against the minimal and maximal measurements of their bones (Fig. 3 A, B). All of those data — based on large series of individuals — were taken from literature (FERENS, 1967 ; TOMEK, in prep.). The graph of minimal versus maximal values of bone and wing parameters delivers four points so, each species is covered by one rectangle in the diagram. It should

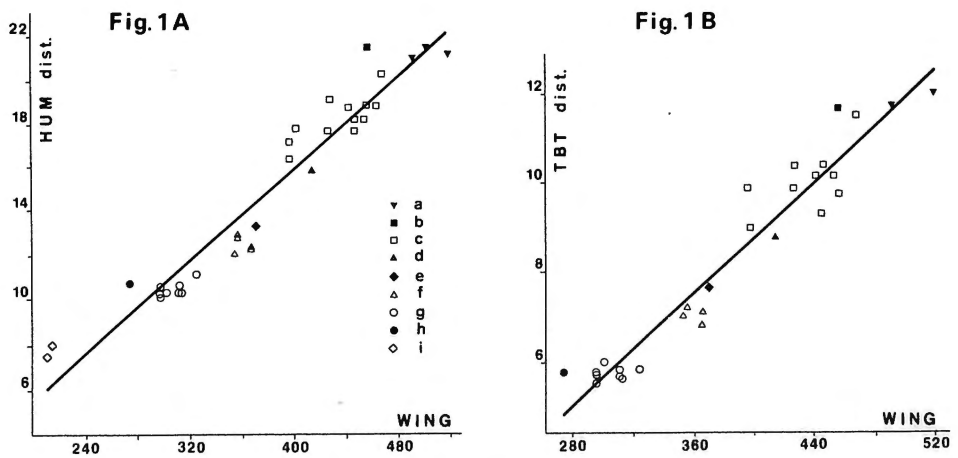
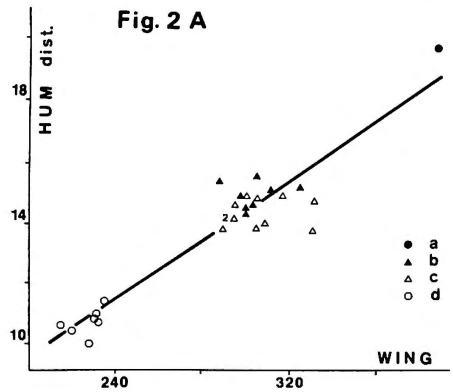


Fig. 1A. — The relationship between the wing length (WING) and the width of the distal part of the humerus (HUM dist.) in the genus *Larus*. Each point indicates one individual. Legend : a — *L. marinus* LINNAEUS, 1758 ; b — *L. hyperboreus* GUNNERUS, 1767 ; c — *L. argentatus* PONTOPPIDAN, 1763 ; d — *L. californicus* LAWRENCE, 1854 ; e — *L. delavarensis* ORD, 1815 ; f — *L. canus* LINNAEUS, 1758 ; g — *L. ridibundus* LINNAEUS, 1766 ; h — *L. pipixcan* WAGL, 1831 ; i — *L. minutus* PALLAS, 1776. Statistics : reduced major axis a = −5.61 ; b = 5.37. Correlation coefficient r = 0.97

Fig. 1B. — The relationship between the wing length and the width of the distal part of the tibiotarsus (TBT dist.) in the genus *Larus*. Legend as in Fig. 1A. Statistics : a = −4.15 ; b = 3.24 ; r = 0.97



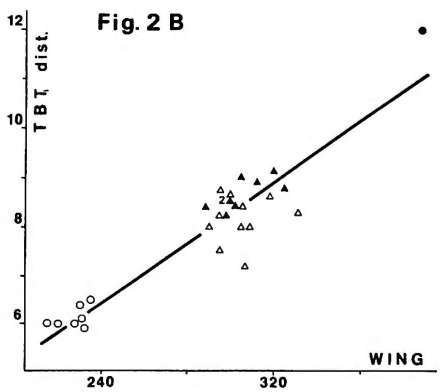
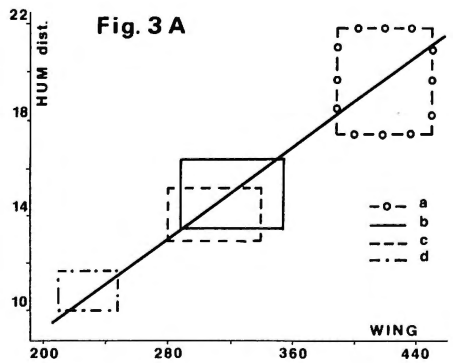


Fig. 2A. — The relationship between the wing length and the width of the distal part of the humerus in the genus Corvus. Each point indicates one individual. Legend : a — *C. corax* LINNAEUS, 1758 ; b — *C. corone* LINNAEUS, 1758 ; c — *C. frugilegus* LINNAEUS, 1758 ; d — *C. monedula* LINNAEUS, 1758. Statistics : a = −1.15 ; b = 5.18 ; r = 0.94.

Fig 2B. — The relationship between the wing length and the width of the distal part of the tibiotarsus in the genus *Corvus*. Legend as in Fig. 2A. Statistics : a = −1.52 ; b = 3.28 ; r = 0.93.



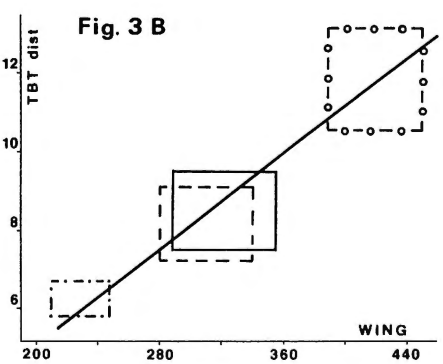


Fig. 3A. — The relationship between the wing length and the width of the distal part of the humerus in the genus *Corvus*. Each rectangle indicates the range of one species. Legend : a — *C. corax* ; b — *C. corone* ; c — *C. frugilegus* ; d — *C. monedula*. Statistics : a = −0.28 ; b = 0.05 ; r = 0.83.

Fig 3B. — The relationship between the wing length and the width of the distal part of the tibiotarsus in the genus *Corvus*. Legend as in Fig. 3A. Statistics : a = −0.98 ; b = 3.02 ; r = 0.82.

be remembered, however, that both variables (the wing and the bone measurements) are normally distributed. If we plotted the two variables against each other in a large series of individuals, we assume that we would get a figure of an elliptic shape for each species. However, due to the lack of numerous

individual measurements and for the sake of simplicity, each species is covered by one rectangle (not ellipse) in the diagram. Data from literature allow us to draw rectangles of horizontal and vertical sides, *i. e.* not slanting in any way. The combinations of minimal and maximal values (*i. e.* 4 vertices of each rectangle for all species studied) were used to calculate the reduced major axis and the correlation coefficient in the same way as in the case of individual points. The high value of the correlation coefficients (3 A : r = 0.83 ; 3 B : r = 0.82) that were found using data from humerus and tibiotarsus, and the similarity of these diagrams (Fig. 3 A, B) to the previous ones (Fig. 2 A, B) indicate that the method can be used for further investigations. The area covered by individual points of each species in Fig. 2 A and B corresponds well with the area and the arrangement of the rectangles in Fig. 3 A and B. Although the correlation coefficients calculated for the vertices of the rectangles (Fig. 3 A, B) are somewhat smaller than the correlation coefficients calculated for individual points (Fig. 2 A, B), they are still statistically significant. In other words, simplified data (rectangles) can be nearly as good as individual measurements (points) in determining the correlation of the wing length and the bone dimensions. So, we compared the minimal and maximal measurements of the wing lengths (taken from literature) with minimal and maximal values of different measurements of various bones (cited in literature and taken from specimens of ISEA) in several groups of birds. The results are illustrated by means of rectangles in separate diagrams. In the case when only one skeleton (*i. e.* one bone measurement) of the species given was available, it is represented by a line segment indicating the minimal and maximal wing length. Single individuals were marked with separate points.

RESULTS

Fig. 4 shows a relationship between the length of the coracoideum and the length of the wing in European ducks from the genus *Anas* (CRAMP and SIMMONS, 1977 ; WOELFLE, 1967). All the rectangles lie approximately on the reduced major axis ; only one of the Mallard protrudes partially above the line. It is connected with the differentiation of the size of drakes in this species. It also may have happened that in the collections studied by WOELFLE (1967) some domesticated (bigger) drakes were included among the wild specimens. Probably due to this fact, the correlation coefficient is a bit lower (r = 0.70) than in the case of *Corvus* and *Larus*. It is also worth noticing that the rectangles of the Wigeon, the Gadwall and the Pintail much overlap this : is connected with the fact that these species are nearly the same size.

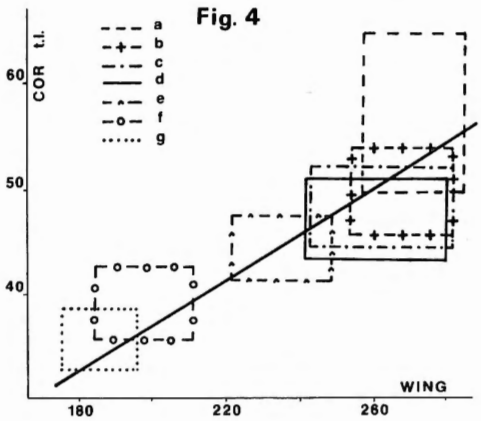
Similar results were also obtained when we compared the length of the carpometacarpus with the wing length in the same species of ducks.

The diagram in Fig. 5 depicts a relationship between the length of the humerus and the length of the wing in 5 species of European grouse (data from : CRAMP and SIMMONS, 1980 ; ERBERSDOBLER, 1968 ; KRAFT, 1972). The sexual dimorphism in the genera *Bonasa* and *Lagopus* is so small that both sexes are combined. On the con-

trary, in the genus *Tetrao,* sexes are shown separately. Although 3 various genera are presented in this diagram, all the rectangles lie again on the reduced major axis. The correlation coefficient is very high (r = 0.96).

The comparison of the wing length (CRAMP and SIMMONS, 1977) and the width of the distal articular part of the tarsometatarsus (KELLNER, 1986) is shown in European herons (Fig. 6). Although there are 9 species belonging to 7 different genera and 2 subfamilies, the rectangles lie again more or less on the reduced major axis. Only the rectangle of *Botaurus stellaris* is more aside but in spite of it the correlation coefficient is high (r = 0.88).

Similar comparison was done for two genera of the birds of prey : *Accipiter* and *Circus* (Fig. 7). In this case it is a relationship between the wing length (CRAMP and SIMMONS, 1980 ; DEMENTEV and GLADKOV, 1951 ; ISEA) and the width of the proximal part of the humerus (OTTO, 1981 ; ISEA). These two genera differ considerably in the proportions of these parameters. Moreover, rectangles of *Accipiter* show better linear arrangement than those of *Circus*. It is also reflected in the value of the correlation coefficients (r = 0.96 and 0.62 accordingly). Rectangles of 2 other



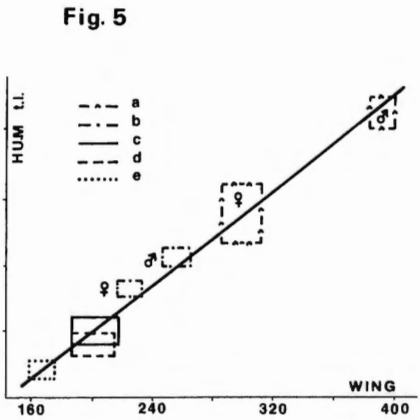


Fig. 4. — The relationship between the wing length and the total length of the coracoideum (COR t. l.) in the genus *Anas*. Each rectangle indicates the range of one species. Legend : a — *A. platyrhynchos* LINNAEUS, 1758 ; b — *A. acuta* LINNAEUS, 1758 ; c — *A. strepera* LINNAEUS, 1758 ; d — *A. penelope* LINNAEUS, 1758 ; e — *A. clypeata* LINNAEUS, 1758 ; f — *A. querquedula* LINNAEUS, 1758 ; g — *A. crecca* LINNAEUS, 1758. Statistics : a = −6.57 ; b = 0.22 ; r = 0.70.

Fig. 5. — The relationship between the wing length and the total length of the humerus (HUM t. l.) in the subfamily Tetraoninae. Each rectangle indicates either the range of one sex or of the whole species. Legend : a — *Tetrao urogallus* LINNAEUS, 1758 ; b — *T. tetrix* LINNAEUS, 1758 ; c — *Lagopus lagopus* (LINNAEUS, 1758) ; d — *L. mutus* (MONTIN, 1776) ; e — *Bonasa bonasia* (LINNAEUS, 1758). Statistics : a = −10.41 ; b = 0.35 ; r = 0.96.

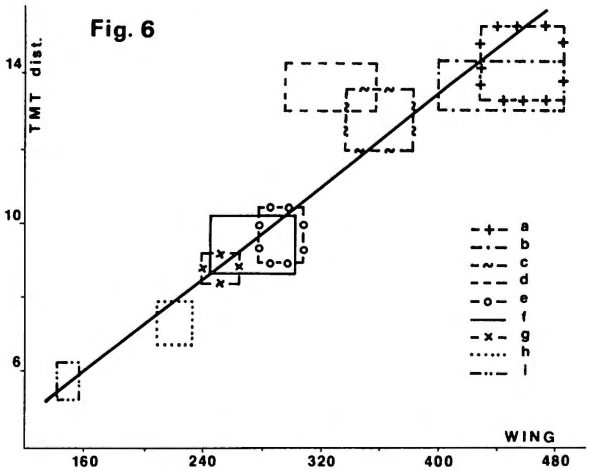


Fig. 6. — The relationship between the wing length and the width of the distal part of the tarsometatarsus (TMT dist.) in the family Ardeidae. Each rectangle indicates the range of one species. Legend : a — *Ardea cinerea* LINNAEUS, 1758 ; b — *Egretta alba* LINNAEUS, 1758 ; c — *A. purpurea* LINNAEUS, 1766 ; d — *Botaurus stellaris* (LINNAEUS, 1758) ; e — *Nycticorax nycticorax* (LINNAEUS, 1758) ; f — *E. garzetta* (LINNAEUS, 1766) ; g — *Bubulcus ibis* (LINNAEUS, 1758) ; h — *A. ralloides* (SCOPOLI, 1796) ; i — *Ixobrychus minutus* (LINNAEUS, 1766). Statistics : a = 1.22 ; b = 3.06 ; r = 0.88.

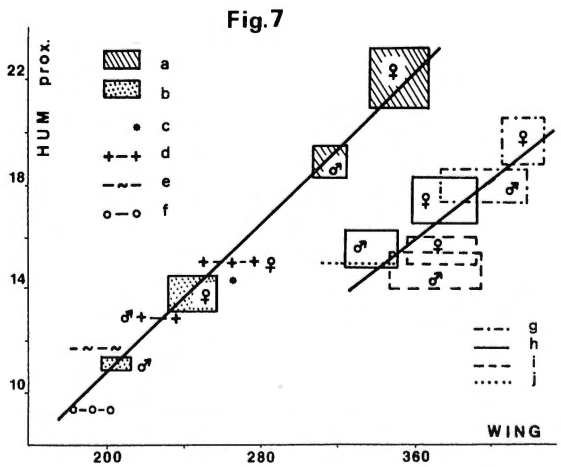


Fig. 7. — The relationship between the wing length and the width of the proximal part of the humerus (HUM prox.) in two genera : *Accipiter* and *Circus*. Each rectangle, line segment or point indicates either the range of one sex or of the whole species. Legend : a — *A. gentilis* (LINNAEUS, 1758) ; b — *A. nissus* (LINNAEUS, 1758) ; c — *A. fasciatus* (VIGORS and HORSFIELD, 1827) ; d — *A. cooperi* (BONAPARTE, 1828) ; e — *A. striatus* VIEILLOT, 1807 ; f — *A. soloensis* HORSFIELD, 1821 ; g — *C. aeruginosus* (LINNAEUS, 1758) ; h - *C. cyaneus* (LINNAEUS, 1766) ; i — *C. pygargus* (LINNAEUS, 1758) ; j — *C. macrourus* (GMELIN, 1771). Statistics : *Accipiter* a = −3.68 ; b = 7.25 ; r = 0.96. *Circus* a = −4.80 ; b — 5.76 ; r = 0.62.

species from another genus (*Buteo buteo* and *B. lagopus*) would be situated between the reduced major axes of *Accipiter* and *Circus*. For the sake of simplicity it was not shown in Fig. 7.

The species of the genus *Falco* serve as the last examples of our investigations (Fig. 8 and 9). The length of the wing (CRAMP and SIMMONS, 1980 ; FRIEDMANN, 1950 ; BROWN *et al.*, 1982 ; ISEA) was compared with the width of the distal parts of the tibiotarsus and the humerus (SOLTI, 1981 ; ISEA ; KUROCHKIN, *in lit.*). In both cases the correlation coefficient is high (tibiotarsus : r = 0.89 ; humerus : r = 0.88). It is worth noting that rectangles of *F. tinnunculus, F. subbuteo* and *F. rupicoloides* are arranged in a different way to each other in Fig. 8 (tibiotarsus) than in Fig. 9 (humerus).

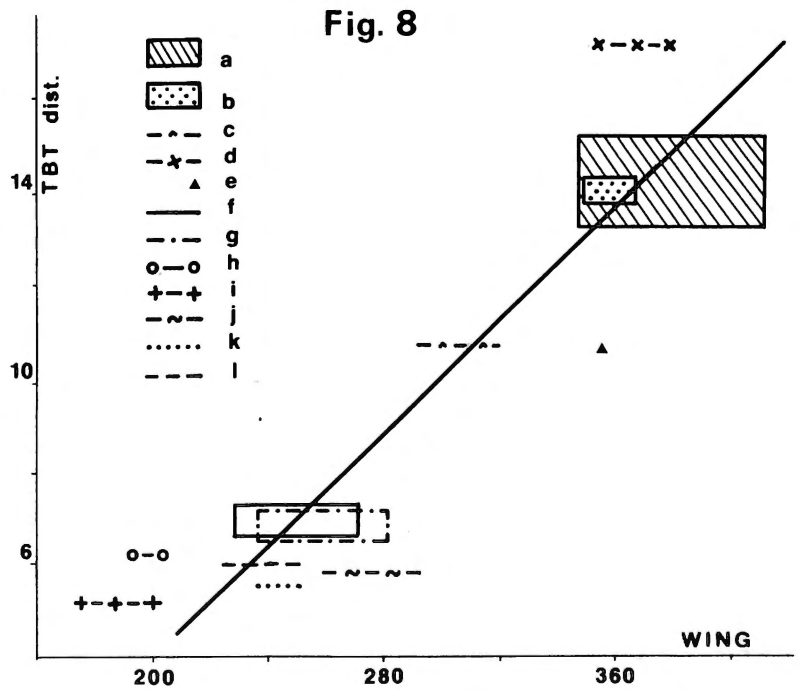


Fig. 8. — The relationship between the wing length and the width of the distal part of the tibiotarsus (TBT dist.) in the genus *Falco*. Each rectangle, line segment or point indicates either the range of one sex or of the whole species. Legend : a — *F. cherrug* GRAY, 1834 ♂♀ ; b — *F. peregrinus* TUNSTALL, 1771 ♀ ; c — *F. peregrinus* ♂ ; d — *F. rusticolus* LINNAEUS, 1758 ♂ ; e — *F. berigora* VIGORS and HORSFIELD, 1827 ♀ ; f — *F. subbuteo* LINNAEUS, 1758 ♂♀ ; g — *F. tinnunculus* LINNAEUS, 1758 ♂♀ ; h — *F. columbarius* LINNAEUS, 1758 ♂ ; i — *F. sparverius* LINNAEUS, 1758 ♂ ; j — *F. rupicoloides* SMITH, 1830 ; k — *F. vespertinus* LINNAEUS, 1766 ♂ ; l — *F. naumanni* FLEISCHER, 1818 ♂. Statistics : a = −8.20 ; b = 0.06 ; r = 0.89.

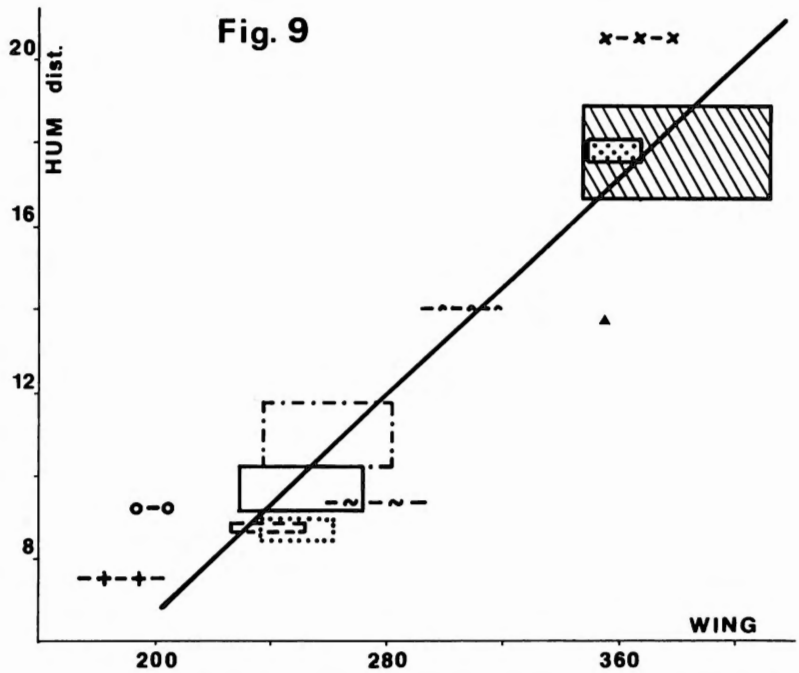


Fig. — 9. The relationship between the wing length and the width of the distal part of the humerus (HUM dist.) in the genus *Falco*. Legend : a-j as in Fig. 8 ; k — *F. vespertinus* ♂♀ ; l — *F. naumanni* ♂♀. Statistics : a = −6.53 ; b = 6.60 ; r = 0.88.

DISCUSSION

The size of a bone fragment depends on the size of a bird, its biology and also on skeletal proportions (BOCHEŃSKI, 1989).

The relationships between the wing length and the bone measurements described above work to a various degree in different groups of birds. It seems for instance that the groups consisting of species differing in the shape and the length of wing (feathers) — due to their sedentary or migratory behaviour — may not show such relationships or their relationships may be weaker. Unfortunately, there are no osteometric data to prove it. Such differentiations in the shape and length of the wings are observed *e. g.* in the genus *Oriolus* (SCHÜZ, 1971 after KIPP, 1936) or even in the subspecies of *Zonotrihia capensis* (VAN TYNE and BERGER, 1959 after CHAPMAN, 1940).

If the above statement is right, shortening primaries (no changes of wing bone sizes) in sedentary birds should shift their rectangles to the left side of the reduced major axis. On the contrary, lengthening of the wing in migratory birds should shift their rectangles to the right side of the axis. The arrangement of rectangles and line segments representing partial and short distant migrants like : *Botaurus stellaris*

(Fig. 6), *Falco rusticolus, F. columbarius* and *F. sparverius* (Fig. 8 and 9) as well as the long distant migrant — *Circus pygargus* (Fig. 7) may support this theory. On the other hand, the data arrangement of sedentary *Falco berigora* and *F. rupicoloides* (Fig. 8 and 9) and a long distant migrant *Circus macrourus* (Fig. 7) does not agree with it. It should be emphasized, however, that in all the cases mentioned above, the rectangles, line segments and points were not very far from the reduced major axis. More or less linear arrangement can be seen in all the diagrams.

The arrangement of the falcon rectangles in relation to the reduced major axis (Fig. 8 and 9) was also compared with the division of the genus into subgenera (WOLTERS, 1975). It appeared that the arrangement of rectangles does not reflect the systematic division based on many characteristics.

The relationships between the wing length and the length of leg and wing skeletons were found in birds of similar biology — *i. e.* in European gulls (DINNENDAHL and KRAMER, 1957). Although the relationships of single bones and the wing length were not studied by them, the results were similar to those obtained by us in the case of the length of the coracoideum in ducks (Fig. 4) and of the humerus in grouse (Fig. 5). However, bone fragments are far more numerous than whole bones in fossil and recent bird remains. The present results indicate that well-defined measurements of bone fragments are also good for studies of proportions.

Our results only indicate the problem. However, the examples of allometric dependencies show that scaling features are essential for identification of bird bone fragments. They lead to the following practical conclusions :

1. The length of wing — characteristic for each species, dependent on the length of full grown primaries and commonly cited in literature — is, to a various degree, correlated with measurements of various parts of the skeleton (wings as well as legs).

2. The correlation of one part of a bone with the wing length is similar in species belonging to a genus or even to a family provided that the birds have the same or very similar body plan. Such a correlation may be presented by a linear arrangement of points or rectangles which may be used for calculation of a reduced major axis.

3. Having examined a relationship of the wing length and a given bone measurement (depending on the fragment which is to be identified) in a certain group of birds (based even on a part of species belonging to the group), one can estimate the wing length on the basis of the size of the bone fragment. It can be simply read from the axis on which wing length is scaled. This, in turn, leads to the identification of the species, or at least helps us to reduce the number of possibilities.

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