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A POINT OF VIEW

FEMALE CHOICE, SECONDARY EFFECT OF « MATE CHECK »? A HYPOTHESIS

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Abstract. A new hypothesis is formulated to explain the diversity and the range of complexity of secondary sexual characters (SSC). It is based on the observation that in many animal groups an important somatic radiation took place but the SSC remained fairly uniform and their complexity low, while in some other well-studied groups it can be shown that, apparently at a later stage, complexity increased dramatically while somatic morphology remained stable. SSC are therefore hypothesised to be linked to hidden (behavioural), but crucial traits that have been acquired in the last steps of the evolution of the taxon. The mating process is postulated to guarantee the presence of these characters. During this process the « mate is checked ». The reason for this mechanism is hypothesised to be the avoidance of the loss of crucial behavioural adaptations through deleterious mutations. The hypothesis might explain why taxa with a flexible checking system (e.g. stridulation, nuptial dance) are more speciose than those using only morphological clues which may be more limited in complexity and variation. Systems that allow larger variation without compromising the survival of the adult male will allow a wider radiation. Since complexity of SSC is hypothesised to be correlated with specialisation, animal groups with smaller species can be expected to have more complex SSC. Female choice is presumed to be a secondary effect of the « mate check » mechanism. The former only operates in optimal habitats where a wide range of the signal strength of the male is to be expected. In marginal habitats (sinks) it is likely to be insignificant because both female coyness and range of male signal strength are assumed to drop. It is precisely in sinks where speciation will occur when behavioural adaptations, consolidated by SSC, allow more efficient use of underexploited resources. Therefore, in contrast to female choice, mate check is viewed as a stabilising mechanism.

Key words : Araneae, cichlids, complexity, marginal habitats, niche pressure, secondary sexual characters, sexual selection, sinks, sources, specialisation, speciation.

INTRODUCTION

The purpose of sex is currently considered to be an insurance for rapid adaptation under changing environmental conditions. It would appear, however, that this apparently well-established theory might have to be abandoned or at least modified, in the light of new insights (OTTO & MICHALAKIS 1998, and references therein). ZEYL & BELL (1997) provide convincing data which indicate that the real advantage of sex is the elimination of deleterious mutations. Some of the hypotheses concerning sexual selection, in particular Fisherian selection, have also acquired the theory status. Since they are probably less well established

than the former, there is reason to consider that status as premature. The alternative mechanism here presented protracts the direction taken by the hypothesis of ZEYL & BELL (1997).

Sexual selection was for the first time formulated by DARWIN (1859, 1871) and was recognised as one of the driving evolutionary forces. Sexual selection has many aspects: sperm competition, endurance rivalry (lek behaviour) and coercion, but male contests and mate choice are doubtless the mechanisms that have received most attention as they are supposed to be the most widespread mechanisms in this respect (ANDERSSON & IWASA, 1996). We will focus on the latter in the present paper.

As far as mate choice is concerned, a remarkable and well known hypothesis is that of FISHER (1930) known as the « runaway process ». According to that model sexual dimorphism is a result of sexual selection *per se* : females choose males for the sake of their ability to stimulate the female which results in a self-reinforcing process, permanently increasing the extent of the secondary sexual characters (SSC: since the terms «primary» and «secondary» sexual characters have led to confusion in the past – see ARNQVIST1997 – SSC is here used as any character, apart from the gonads, that contributes to sexual dimorphism). The reasoning behind this hypothesis is that the female's preference for a certain type of male is heritable and will be similar in her daughters. Fisherian selection is still often invoked to explain sexual dimorphism. Another model that is rapidly gaining influence is the «good genes» hypothesis (also called « handicap » or « indicator » model) which assumes a link between the quality of the male ornament and his overall physical fitness. In other words, the stronger the male's signal, the higher its fitness. This hypothesis was also proposed by FISHER (1930), reformulated by ZAHAVI (1975, 1987) and translated into a population genetic model by GRAFEN (1990). Females selecting a strong male signal would thus be guaranteed higher survival rates of their offspring. A third model that receives increasing attention is a modification of the indicator model called the «revealing indicator model» which expects a certain quality of the male (e.g. resistance against parasites) to be reflected in the male's ornament (HEYWOOD, 1989). A further model is the « direct benefit » hypothesis (KIRKPATRICK 1985, 1987) which assumes that females tend to mate with males that maximize female fecundity. Species recognition is yet another explanation for mate choice and one of the earliest hypotheses to explain it: SSC are assumed to have evolved in order to avoid interspecific mating. Finally, the « sensory exploitation » model (RYAN 1990, RYAN *et al.* 1990, RYAN & KEDDY-HECTOR 1992) assumes that males take advantage of the female sensory capabilities that antedate the origin of the sexual selection dynamic and the evolution of the male and female traits is thus decoupled. It is evident that some of these mechanisms overlap and have been combined to explain particular cases of female choice.

The present paper formulates a new hypothesis that focuses on the quantity of information that is transferred from the male to the female during the entire mating process and is assumed to be a mechanism to avoid deleterious mutations which might easily affect fine-tuned behavioural adaptations.

THE HYPOTHESIS

The nucleus of the hypothesis is as follows :

In order to avoid the loss of crucial behavioural adaptations by deleterious mutations that would compromise survival, incipient species develop secondary sexual characters linked to these adaptations ; the mating process is thus construed so that it guarantees the presence of these characters, and during this process the « mate is checked ». This implies that SSC transfer a certain amount of information related to the species' degree of specialisation and that there is no necessity for a causal relationship between the behavioural or somatic trait and the SSC that is linked to it. It has the important implication that female choice is a result of the presence of SSC and not the cause of their origin ; whereas female choice is considered to increase the rate of evolution, mate check on the contrary is proposed to have a stabilising effect.

The background to the idea is the fact that parts of the genome are more susceptible to mutations than others. As mutations are more likely to occur in such an active zone, the chance for occurrence of exactly the deleterious mutations that compromise newly acquired adaptations is high . « Mate check » can be considered a mechanism to prevent mates with such deleterious mutations taking part in the reproductive process.

Although the present paper does not intend to formulate a genetic background of the mechanism, one of the possibilities is the implication of pleiotropy. The information that is transferred to the female by morphological or behavioural sexual traits, operating during courtship and mating, has to be genetically linked to the hidden characters. MAYR (1963) argued that SSC are selectively neutral but influenced by genes that code for selectively important traits. EBERHARD (1985) refuted this hypothesis as it does not explain why (a) only SSC and not somatic characters tend to be affected and (b) primary genitalia are unaffected when sperm transfer is mediated only by secondary genitalia. ARNOLD (1973) proposed a similar hypothesis but assumed that genitalic characters are not neutral but assure species-specific matings by a lock and key mechanism. Whereas EBERHARD's (1985) refutation of the lock and key hypothesis is extensive and convincing, his arguments concerning pleiotropy arè not. It should be obvious that just like other characters, linked traits are subject to selection and will disappear when they are disadvantageous. SSC as well as somatic characters might be affected by pleiotropy linkage, but when these links are not advantageous they are bound to disappear, possibly together with the characters themselves.

FITNESS : THE BEHAVIOURAL COMPONENT

Apart from on its morphological adaptations, the survival of an individual largely depends on its behavioural adaptations. In order to be successful, it has to act in the right way : it should prefer the right habitat, be active at the right time (in the right season, at the right time of the day), show those food preferences that enable it to obtain enough resources and, importantly, behave in such a way as to avoid predators.

As these kinds of adaptations, although crucial for survival, often have no directly obvious morphological expression, the hypothesis suggests that information about their presence is conveyed by SSC (see Fig. 1).
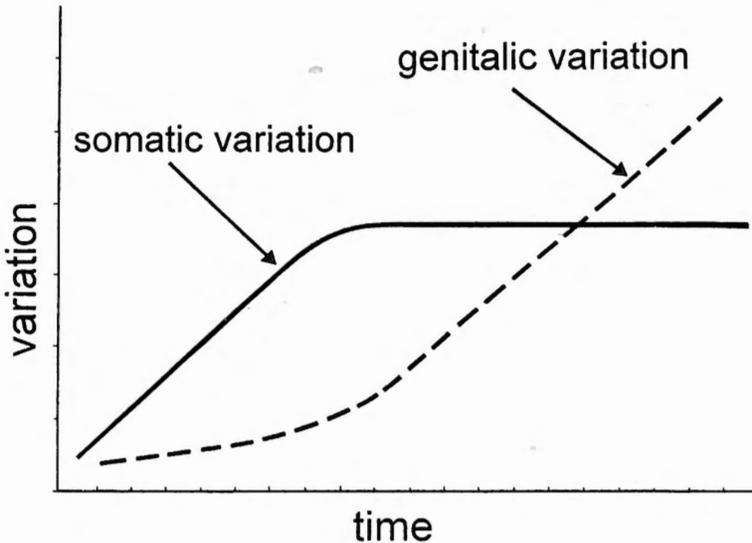

Fig. 1. – Fluctuation of somatic and genitalic variation in time within a particular animal group: a period of strong somatic radiation and relative genitalic stability, is followed by of period of rapid genitalic radiation and stable somatic patterns.

It is evident that in the course of time, as niches become narrower, all these preferences undergo a tendency towards higher specialisation. As the number of species increases and mechanisms of protection of possible resources, plants as well as animal prey items, improve, the need for specialisation undergoes a parallel increase. Together with that increasing specialisation, the amount of information transferred during (pre)mating has to increase. It is clear from phylogenies for certain invertebrate groups (e.g. Araneae, *Tenedos*: JOCQUÉ & BAERT 1996, *Storena*: JOCQUÉ & BAEHR 1992, BAEHR & JOCQUÉ 1994, Lycosoidea: Griswold 1993, Diptera: Mycetophilidae: SÖLI 1997, Pipunculidae: DE MEYER 1995, 1996) that the complexity of genital structures has increased considerably in the course of evolution. This has been explained as a random phenomenon greatly dependent on incidental preferences of females. In the present hypothesis it is argued that when particular characters, behavioural and perhaps morphological, develop, they have to be exteriorised by parallel genital traits. A famous example is that of black-headed male sticklebacks which exhibit flight reaction from particular large fish predators, such reaction being absent in the red-headed males that mainly occur in habitats where these predators are absent. The development of the flight reaction is exteriorised by the development of the appearance of a black head in the male during the breeding season (MCPHAIL, 1969). This enables the female to «verify» that a particular male has acquired the flight reaction. Females that choose red-headed males choose a partner that does not have this reaction.

Another example might be found in spiders. In species of the genus *Hortipes,* a recently described genus of African Liocranidae (BOSSELAERS & LEDOUX, 1998), I observed that the males from South African mountain forest species have simple palps whereas a somatically almost identical species from West African lowland forest has much more complex palps although it is not clear yet what particular palpal character is involved. The circumstances in which both these animals live is extremely different, mainly in the numbers of predators encountered (ants, see for instance JOCQUÉ 1984). Observation of live males showed that when disturbed, lowland males jumped about as far as ten times their body length whereas highland males just ran for a short bout not exceeding four times their length. We assume that the flight reaction acquired by the lowland spiders is exteriorised by one of the characters in the (much) more complex palp. In the same genus another example of behavioural adaptation that might be linked to palpal morphology can be found. All representatives of this genus live in the litter layer of evergreen forest except one which was found mainly in grassland surrounded by forest in Natal. That particular species is part of a large clade with many closely related species which are difficult to separate. Although it is evidently part of that clade, it differs from the other species by an unusually large number of autapomorphies. The apparently dramatic change in behaviour that enables this species to live in grassland rather than in forest is assumed to be accompanied by a clear shift in palpal morphology. How the information could be transferred in such a system is not immediately clear but is likely to be mediated by cryptic female choice (see EBERHARD 1996 for a review) which should then be called « mate check ». Males that do not have exactly the right behaviour and the exact male palpal morphology linked to it, do not succeed in passing sperm in the right way or to the exact spot in the epigyne and are likely to have their sperm rejected in one way or another. It is inevitable that the female genitalia must provide means to select, in some cases cryptically (EBERHARDT 1996, HUBER & EBERHARDT 1997) the characters of the male genitalia. This explains why in the past, the complementary structures of male and female genitalia have lead to hypotheses such as « lock and key » and mate « recognition ».

The question arises whether all newly acquired traits have to be exteriorised by sexual traits. The answer is probably no. Those characters that have a conspicuous morphological expression probably do not require sexual exteriorization as they already play a role in the initial recognition between the sexes and are likely to be automatically checked during the onset of courtship. However, if a particular somatic adaptation is only meaningful in combination with specialised behaviour, it is likely that the behaviour needs back-up of a particular SSC.

LIMITS TO EXTERIORISATION

It is evident that every system that exteriorises specialisation reaches a limit of complexity when niches become narrower and the species more specialised. It is therefore understandable that in many animal groups traits other than purely genital secondary sexual ones have developed. If the sexual organs have reached a limit of complexity, due to structural or evolutionary constraints (see HORNE *et al.* in press), other traits can be added to accompany increased behavioural or morphological complexity. The possibilities

in this respect have been reviewed mainly by EBERHARD (1985). It should be noted though, that certain developments are easily understood in the light of necessary increasing complexity of sexual organs. In spiders for instance, the evolution of the epigyne has increased the possible complexity for the male palp tremendously (examples of haplogyne versus entelegyne). This would mean that the success of a group and its proneness to radiate is highly dependent on the flexibility and the variability of its mating system, which has been called a « copulatory module » (MARTENS, in press). The success of cichlid fishes in great lakes (FRYER 1991, KAUFMAN *et al.* 1997) may at least partly be explained by fine-tuning of male characters (colour for instance) during courtship. These small colour variations in courting males often appear to be the only morphological differences (SNOEKS 1991, SEEHAUSEN & VAN ALPHEN, 1998) in species that occupy very specialised niches. These however, must be considered the crucial information for the matching females. Males of specialised grazers for instance, advertise their specialised grazing behaviour by their colour which is the only means female have to check the presence of this crucial character. Males with slightly different colour may advertise different grazing behaviour. Although the original meaning of this system is here assumed to check the genetic quality of the male, recognition may be a secondary advantage in mixed populations of strongly related species.

Traits other than morphological ones offer more possibilities for variation and are often those that do not compromise the viability of the males, thus facilitating further specialisation than purely morphological traits would do. Visual, auditive, chemical and even electrical (mormyrid fishes) stimuli offer probably many more possibilities to transfer information than would purely morphological ones. In spiders and insects with good eyesight a good deal of the courtship and thus mate check, is based on visual stimuli. It is probably not a coincidence that precisely those families that rely on such information transfer are among the most speciose (Salticidae, Lycosidae in spiders; Hawaiian Drosophilidae and many other groups in Diptera; Cichlidae in Pisces; birds as a whole).

Auditive information transfer is probably even more efficient as the « sender » can remain concealed while transferring messages. Perfect examples are Passeriformes among birds (with the development of the syrinx), frogs and toads among Amphibia, Orthoptera and Homoptera among the insects. Another possibility is the use of pheromones which have advantages similar to auditive messages. The extremely low concentrations at which these chemicals can be used allow very low population densities. It is clear that chemical information transfer is often used as a complement to tactile and auditive systems: e.g. presence of glands in cephalothorax pits in male Linyphiidae (Araneae) obviously serve to transfer information during copulation. In this family, tactile, auditive and chemical stimuli are used in combination, which is assumed to be an apparent consequence of the narrow niches in which these spiders live, since up to 50 species may occur in the same macrohabitat. Animal groups that use these kinds of information transfer during courtship are likely to be much more flexible as they have a much larger array of possibilities than those that use purely morphological traits and radiations are therefore to be expected in these groups. The efficiency of sex linked information transfer might be crucial in groups that have similar ecological possibilities. The success of Araneidae (Araneae) in comparison with Uloboridae, both of which make orbwebs but with different sticky systems, may

be due to the more flexible SSC of the former rather than to the inferior capturing system of the latter.

PHYSICAL ADAPTATIONS AND FEMALE CHOICE

From the above it is clear that the hypothesised «mate check» is an all or nothing mechanism. The selection by the mate depends entirely on the presence (quality) of the SSC. The SSC, however, are themselves subject to variation and the strength of the SSC varies along a quantitative gradient.

In contrast to behavioural adaptations, physical adaptations can be deduced in the first place from the somatic quality of a partner, its size and strength, in the second place from the quantitative expression of its SSC: their size, intensity of their colour, song intensity etc. If verification of the presence of these characters is important, females may develop an a posteriori attraction to these characters and should thus be stimulated by their presence. It is then evident that the more developed is the secondary sexual trait, the stronger will be the signal and the easier will be the verification by the female partner. It is therefore only reasonable that females, if given the opportunity, choose the male with the strongest signal. Female choice could therefore be considered a secondary effect of «mate check».

Secondary sexual characters are likely to vary quantitatively, just as for instance the size of an (r-selected invertebrate) organism does. The stability of size is a very similar phenomenon: although larger females have an advantage as larger size enables larger clutches, size remains stable around a certain average, simply because there is a strong feedback as size tends to be a significant adaptive trait, and small females may have a strong advantage in adverse conditions. Likewise, secondary sexual characters may vary to a certain extent, but, for the same reasons, fluctuate around a fixed average. The strength of the male signal exerted by SSC is a result of genetic and phenotypic variations (Fig. 2) as are size and the quality of other morphological characters. It is easily understood that in a purely theoretical condition, when all circumstances are optimal (here shown as optimal habitat quality) all variation is due to the genetic component. When however, the quality of the environment decreases, the phenotypic component increases in importance. The variation as a whole is the sum of both genetic and phenotypic variation.

The result of this phenomenon, the quantity aspect, which explains the cases in which brighter, larger etc. characters are favoured as they reflect the physical component of fitness, has been misinterpreted as the driving force behind the development of SSC. A weakness of these hypotheses is indeed that they have rarely made a clear distinction between the qualitative (presence) and quantitative (expression) aspects of SSC and consider their acquisition a result of exaggerated quantitative traits. In practice it is extremely difficult to separate the phenotypic and genotypic component of the quantity of SSC. For practical reasons, observations and experiments are mainly carried out in near optimal conditions, and thus it is likely that the observed heritability is biased since it may be expected to be higher than average in these conditions. It is therefore important that the entire population is considered, including those individuals that live in marginal conditions.
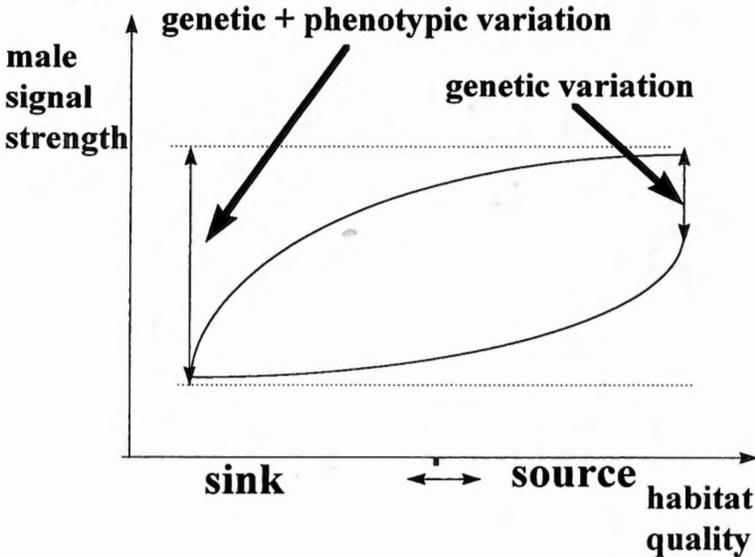
Fig. 2. – Variation of intensity of the signal of male secondary sexual characters. In a purely theoretical condition, when all circumstances are optimal (here shown as optimal habitat quality) all variation is due to the genetic component. When however, the quality of the environment decreases, the phenotypic component increases in importance. The variation as a whole is the sum of both genetic and phenotypic variation. It is assumed here that speciation events occur in the lower reaches of the environmental gradient and not in its upper part as implied by the female choice hypothesis.

If the development of an SSC is the result of runaway evolution or of an indicator mechanism, there should be a very unstable situation. One of the predictions of female choice (EBERHARD 1985) is indeed that SSC should be highly variable as males would be involved in an arms race to acquire SSC with as strong a signal as possible. However, HUBER (1996) and mainly EBERHARD et al. (1998) have shown that secondary genitalia and other SSC in insects and arachnids tend to be more stable than somatic characters. It thus appears that, at least in organisms with what they call « cryptic female choice » (EBERHARD 1996), the mechanism involved is a precise « mate check » rather than female choice. In organisms with « overt choice » (in contrast to « cryptic choice »), SSC do indeed strongly fluctuate around a certain average. Here again, a distinction should be made between quality (presence) and quantity (expression) of SSC. Once a new SSC (a new « quality ») is acquired, the strength of its signal will fluctuate (quantitatively) around a certain average and remain stable around that average. This is in concordance with the punctuated equilibrium theory (ELDREDGE & GOULD 1972, GOULD & ELDREDGE 1986), which states that most species tend to be stable for a relatively long period in geological time. So female choice must be considered the result of the evolution of SSC and not their origin. The presence of an SSC increases the possibility for females to choose between signals of different strength. In cases with overt choice, SSC indeed provide much better possibilities for

sexual selection than purely somatic characters alone would do, since the degree of expression of SSC, hence their signal strength, appears to be quantitatively more prone to variation than other characters. MØLLER & POMIANKOWSKI (1993) provide evidence that the patterns in fluctuating asymmetry for SSC, differ from those in somatic traits and that the former show much higher levels of fluctuating asymmetry.

The difference between the two types of choice, overt or cryptic, prompts another comment: in organisms that apply overt choice, primarily vertebrates, and on which most of the literature on sexual selection is based, the influence of the SSC themselves may often be obscured, mixed as it is with many other influences especially the mate's complex behaviour. Particularly in vertebrates, behavioural adaptations cannot always be considered as hidden. During the intensive interaction between mates, even outside the mating process, females may be able to interpret the adaptive quality of the male's behaviour so that the need for exteriorisation is lower. In animals with cryptic choice on the other hand, contact between mates is often very superficial and of very short duration if occurring at all. A perfect example are those taxa in which there is no copulation and sperm transfer is mediated by spermatophores, that may vary to a great extent (e.g. Pseudoscorpiones; Amblypygi) (WEYGOLDT 1969, WEYGOLDT & HOFFMANN, 1998). In these circumstances there is no appreciation possible of the male's behaviour and any information about it is has to be transferred via the spermatophore. Similar considerations apply to most invertebrates that only meet during copulation. For these reasons, the importance of « mate check » will be much more difficult to demonstrate in « higher » animals than it will be in invertebrates.

ARNQVIST (1998) recently argued that complex SSC are obviously the result of female choice as monandrous invertebrates appear to have much more simple SSC than do polyandrous species. The greater possibility for the females of the latter category to really choose, is supposed to increase the tendency to radiation. Apart from the fact that this mechanism cannot explain the evolution of complex spermatophores, the main question in this context has not been formulated. Since mating is a very costly activity requiring a high amount of time and energy, and considerably increases the risk of being detected by a predator, it should be questioned why some species do mate more than once while others do not? The answer may be found precisely in the correlation detected by ARNQVIST (1998), which also works the other way round. If SSC become more complex with specialisation, it is conceivable that the risk of unsuccessful matings increases accordingly. Multiple matings would therefore become a necessity to keep the risk of remaining unfertilised low, and polyandry could then be considered as an inevitable drawback of high specialisation.

MATE CHECK AND THE ENVIRONMENT

It has been shown several times that the quality of male ornaments is linked to breeding success and to survival rate of the offspring (already mentioned by WALLACE 1889, ANDERSSON 1994:26, WILLIAMS 1975, 1992, PETRIE 1992, 1994, MØLLER 1994).

It may be questioned, however, whether the observed survival rate is only a question of correct mate choice. Little attention has been paid to the effect of the environment on the

incidence and thoroughness of mate choice. In marginal habitats or in bottle-neck situations it is very likely that females are much less fastidious (Fig. 3) than they would be in more advantageous circumstances. The risk of remaining uninseminated might increase considerably in cases of low population density. Time investment in courting may also become risky if resources are scanty and time needed to acquire them is high. It is therefore not unlikely that females which have grown up in favourable conditions, are much more choosy and that the high survival rate of their offspring is mainly due to their own condition and less to the quality of the male as advertised by the quantitative expression of the male's SSC.
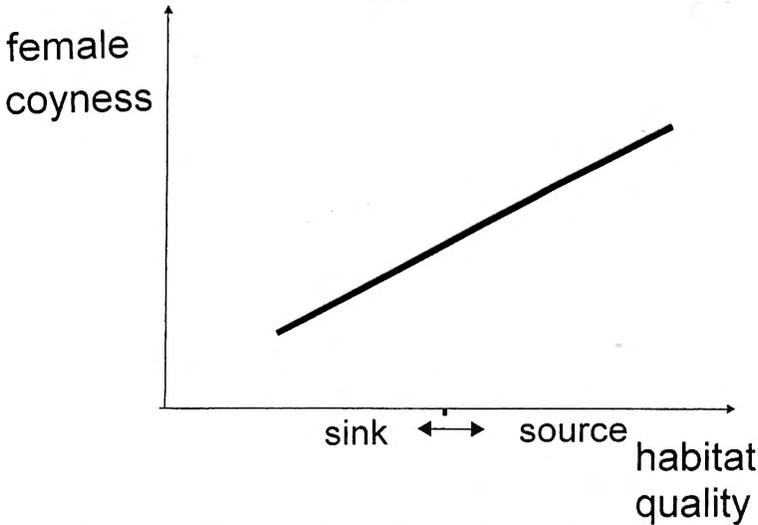

Fig. 3. – Variation of female coyness in respect to the selection of males. In a marginal environment (sink) the female will be less choosy as both the risk to remain unfertilised and the time needed to acquire resources increase and less energy may be invested in courtship-related activities.

Selection for a strongly developed secondary sexual trait does not select for an adaptive character but for the physical fitness component, and there is an increasing amount of evidence that this type of fitness is not heritable (GUSTAFSSON 1986; SMITH 1988; WILLIAMS 1975, 1992).

It may be argued that « mate check » is identical to the « good genes » hypothesis. The main difference between these hypotheses is that mate check is an « all or nothing » (qualitative) mechanism that also works in marginal habitats or bottle neck circumstances whereas « good genes » is a quantitative mechanism in which the driving force is female selection of the strongest male signal. As the strength of the male signal is the result of both genetic and phenotypic influences, the quality of the genes might be masked by phenotypic regression. This means that the « good genes » model proposes a risky mechanism that might jeopardise breeding in adverse conditions. « Mate check » on the other hand remains efficient in any circumstances. Another difference is that « mate check » assumes

a link between particular behavioural characters that have been acquired in the recent evo-lution and the SSC of the taxon whereas «good genes» assumes a random effect, reflect-ing overall fitness. « Mate check » can therefore be expected to generate different levels of complexity whereas « good genes » does not. « Good genes » does not explain the regres-sion of SSC in cases of ecological relaxation nor the rapid decline of species in conditions where « checking the mate » becomes difficult. However, HURST & POMIANKOWSKI (1998) and WILKINSON et al. (1998) provide a new interpretation of the « good genes » model. According to their data, female choice selects a male SSC (in this case long eye span of Diopsidae flies) that is linked with a meiotic drive suppressing gene. Although it is not clear how common the phenomenon of meiotic drive might be, it shows that the idea according to which females assess general male viability is certainly not universal.

Another consequence of the reasoning behind female choice is that speciation would mainly occur at the top end of the habitat gradient, where female choice is highly selective. This hypothesis does not take into account the environmental conditions involved in speciation. It is much more likely that speciation occurs at the lower end of the gradient, in marginal habi-tats or sinks where resources are much less efficiently exploited than at the other extreme. The acquisition of a new (behavioural) character might enable the new species to exploit the resources more efficiently and thus become established. The development of a new SSC that backs up the newly acquired adaptation is hypothesised to be crucial for its consolidation.

For all these reasons it would seem that many of the studies that have tried to show that sexual selection favours the evolution of SSC (e.g. ALATALO et al. 1991 ; PETRIE 1992, 1994) have focused on the wrong aspect. It is also important to note that most of the exper-iments and observations that have studied female choice and its influence on survival of offspring were done in the upper environmental gradient. In this part of the gradient, the genetic component that influences the quantity of SSC is assumed to be higher than the phenotypic component. In the lower reaches of the gradient the latter will be of much higher importance and may help to explain why SSC vary around a certain average.

On the other hand, relevant studies are those that have linked changes in the set of SSC to radiations or to changes in the environment. The studies on cichlids (Pisces) in the African great lakes are well documented in this respect (SEEHAUSEN et al. 1997, GALIS & METZ 1998).

An obvious question that arises when one examines the present hypothesis is the fol-lowing : if the mechanism has evolved mainly as a system to avoid the loss of crucial behavioural adaptations, would it not be logical to expect that males that have undergone such a deleterious mutation would not survive to adulthood, thus rendering the mechanism meaningless? Populations live in a highly variable environment, both in space and time. Some populations or parts of them unavoidably occur in marginal habitats and, more importantly, they are regularly subjected to periods of environmental stress, witnessed by the frequent extinction of isolated populations (MAC ARTHUR & WILSON 1967). Poorly adapted males might survive optimal conditions and thus take part in the reproductive process, but be rapidly exterminated as soon as conditions become critical. The offspring of females that have accepted a male without the crucial adaptations may thrive as long as the conditions stay favourable, but go extinct when they become adverse. The offspring of those females that have checked their mates more efficiently, will have a much higher chance for survival in bottle neck conditions (WIENS 1979).

Another problem for the «mate check» hypothesis is the following. If checking the hidden adaptive qualities of the male is of primary importance for the female, why is it not as important for the male to check the female? The answer to this question should not be fundamentally different from the one proposed in other hypotheses for the evolution of complex animal SSC (see ANDERSSON 1994). The competition for females between males is apparently so strong that it would be a risky strategy for males to build-in a system that restricts the females with which they can mate. Finding a receptive female is likely to be of utmost importance, and restricting the possibilities is likely to be counterproductive in any sense of the word.
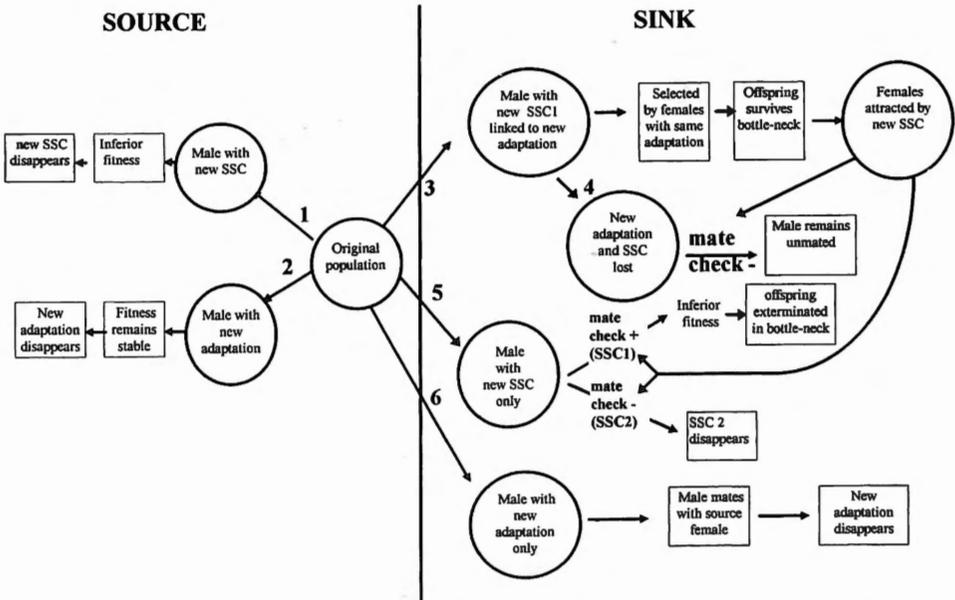

Fig. 4. – A possible scenario of the way in which mate check arises and operates. Circles represent individuals, boxes events and mechanisms. The original population is well adapted to the source situation. New SSC (pathway 1) will increase the males' handicap without compensation in fitness and will soon disappear. New adaptations (pathway 2) have no significant effect on the fitness which remains stable. In the sink situation on the other hand, individuals with a new adaptation are able to exploit resources in circumstances that are marginal for the original population. This is what happens in pathway 3: males with both the new adaptation and SSC1 linked to it, are selected by females with the same adaptation. Their offspring is able to survive the adverse conditions the members of the original population could not. Females become a posteriori attracted and/or stimulated by the new SSC1 of the male. From this stage on, males are checked for the presence of SSC1. In pathway 4, the new adaptation and SSC1 have been lost and males remain unmated since not accepted by mate check. In pathway 5, males acquire a new SSC. If it is SSC1, exactly the same as the one linked to the new adaptation mate check will have a positive outcome but the offspring will not survive an environmental bottle neck situation and disappear. In case it is another SSC, mate check has a negative outcome and SSC2 disappears.

Pathway 6 presents what might happen before the evolution of SSC1 and the connected mate check. Males with the new adaptation mate with source females and the character disappears . Once «mate check» for SSC1 has evolved, this pathway follows the same course as 4.

CONSEQUENCES AND PREDICTIONS

As the complexity of the SSC is hypothesised to be linked to specialisation, one should expect that in similar representatives of a particular guild, the groups with small-sized representatives would have more complex SSC than would representatives of large size. The simple reason is that the smaller the organisms, the higher the habitat specialisation may be. Good examples are Paradoxosomatidae (HOFFMAN & HOWELL 1985), tiny diplopods which have particularly complex genitalia, Linyphiidae (and some other spider families with tiny representatives) which have a large array of SSC on top of very complex secondary genitalia, Membracidae (Homoptera) with complex appendages, Mycetophilidae (Diptera), Passeriformes in birds which have enormous possibilities thanks to the development of the syrinx (RAIKOW 1986, 1988) and barbs (Barbus) and cichlids, mainly in African lakes.

The above hypothesis inevitably results in a time sequence. In the course of time, as niches become narrower and species more specialised, genitalia are assumed to have gone through a parallel evolution of increasing complexity. The organisms that have survived are those that have evolved systems that enable enough information transfer to match the acquired specialisation. It is likely that in certain circumstances (after global disasters, colonisation of underpopulated areas) a certain regression has occurred which may explain instances in which a general decrease in complexity of SSC has occurred.

If we accept that SSC complexity is related to specialisation we may indeed expect a reversible mechanism. If for one reason or another a population undergoes ecological relaxation it should be possible that its sexual characters become equally simple. A few observations point indeed in that direction. KANESHIRO (1983) reports on a case in Hawaiian drosophilids that colonised a new island; courtship in the species is simplified because the receptivity threshold of females is lowered. The same phenomenon was observed in populations of *Drosophila adiastola* that were kept in captivity. After a number of generations the highly specialised species showed simplification of its courtship. In both cases the phenomenon can be explained by a decrease in ecological specialisation paralleled by a decrease of courtship complexity. Hawaiian drosophilid species are highly specialised and each lives on very restricted resources which implies a highly typical behaviour. When individuals are transferred to different ecological circumstances or kept in captivity, resources are bound to be more readily available to them, the species' behaviour less characteristic and complex, and the amount of information to be transferred during courtship equally less complex. In his monumental review of the subject, ANDERSSON (1994) states that «the roles of ecological divergence and sexual selection in these speciations therefore are hard to disentangle». If however, the present hypothesis proves correct, they are not entangled at all but should be perfectly linked.

Another well known example is the occurrence of drabness in island birds. Birds with brightly coloured males often tend to become much more drab after colonising an island. In the light of the present hypothesis this should be understood as the result of the occupation of a less specialised niche. The number of predators may be lower, the food resources more diverse and hence the behaviour of the birds different, and less specialised, from that of the source population. The amount of information to be transferred to the

females is lower and certain secondary traits might be lost. As auditive messages are probably the primary information carriers among sexes (CATCHPOLE 1987) and colourful plumage is only adopted in highly specialised species, it would not be surprising that the first shifts in island colonisers are to be found in the colour of their plumage (GRANT 1965, LACK 1968).

But the reverse is also possible: ecological specialisation might be lost as a result of less efficient mate verification. In some cichlid groups in Lake Victoria, many particularly specialised species disappeared as a result of eutrophication (SEEHAUSEN et al. 1997). In these fishes mate check is supposed to be based mainly on male colour pattern. As the visibility and the possibilities for recognition of the colour pattern decreased with increased turbidity, many specialised species that are here assumed to be maintained by mate check, disappeared. In this example the selection primarily maintains reproductive isolation between closely related, highly specialised species that live in sympatry. However, the mechanism is equally crucial for highly specialised species that live in isolation. Interesting examples in this respect are the studies on cave spiders with complex genitalia (BOSSELAERS 1998, WEISS & HEIMER 1982). The latter authors described two cave spiders with very complex secondary genitalia. They express their confusion about these apparently useless organs as these species live in perfect isolation in different parts of the cave. In the light of the present hypothesis it could mean though, that the complex genitalia exteriorize the strong specialisation connected to troglobitism.

The supposed exteriorization of behavioural characters is likely to be hierarchical. Only those characters that are not automatically linked with the morphology of the species have to be exteriorized. Cichlids that graze algae do not need special SSC as their morphology compels them to do so. However, the behaviour that regulates on what kind of substrate, what depth and in what other circumstances they graze, does need exteriorization. Only behavioural characters that present a shift from the ordinary original behaviour do need exteriorization. Therefore, a system that allows a species to get rid of exteriorization of old characters that are supported by the morphology itself, is particularly flexible and bound to be successful. The statement of Lande (1981) that the evolution of a new SSC may contribute to the decline of an old one must be seen in this perspective.

MATE CHECK AND PHYLOGENY

A possibility to test the present hypothesis is provided by the following: if the hypothesis is correct there should be a correlation between the complexity of the behaviour and the soma on the one hand and that of the genitalia on the other hand. However, in some well studied spider taxa (Araneae Lycosoidea: GRISWOLD 1993, ZODARIIDAE: JOCQUÉ 1990, JOCQUÉ & BAEHR 1992, BAEHR & JOCQUÉ 1994, 1996, JOCQUÉ & BAERT 1996), there is no such clear correlation between somatic and genitalic complexity at generic level. There is evidence that an early split-up into very different somatic patterns (genera) occurred while the SSC remained fairly simple. Afterwards the genera radiated into many similar species in which the somatic pattern remained stable but the SSC became sometimes spectacularly complex (GRISWOLD 1993, JOCQUÉ & BAEHR 1992, JOCQUÉ 1990, BAEHR & JOCQUÉ 1994, 1996, JOCQUÉ & BAERT 1996, BOSSELAERS & JOCQUÉ, in prepara-

tion). This phenomenon may be the result of an initially easy adaptation with large somatic differences which did not need highly sophisticated SSC. Much of the information transfer was achieved simply by recognition of purely somatic characters. Once these many different basic patterns had been established, further specialisation was mainly achieved by behavioural adaptations. This statement would indicate that mate check is in the first place a mechanism to verify behavioural adaptations and that strong specialisation based on highly specialised behaviour is to be backed by increasingly complex SSC.

As pointed out above, the increase in complexity of the male SSC must be seen in the light of mainly behavioural traits as somatic changes are not necessarily accompanied by changes in the SSC. Within a large clade with stable somatic morphology as in the examples mentioned above, the increase in complexity of the SSC is supposed to be an answer to increasingly specialised niches. As these become narrower with time, it should be assumed that the species with simple SSC, the most plesiomorphic taxa, have gone extinct or become very rare and should only be found in relict habitats. The most derived taxa on the other hand should also be rare as they are supposed to be highly specialised and only occur in very narrow niches. The most widespread species should therefore be found in the intermediate taxa. I therefore assume there must be a relationship between the degree of apomorphy (derivedness) of the SSC and the distribution area of the species.

MATE CHECK AND SPECIATION

Apart from speciation by genetic drift which is proven for instance by the existence of ring-species, a probably more common type of speciation occurs by the acquisition of new traits, behavioural or morphological novelties. That kind of speciation is bound to happen in « sinks » (marginal habitats) (DIAS 1996) where the selection by females is less severe. A mutation that allows more efficient exploitation of the sink's resources, through a change of morphology and/or behaviour, is exteriorised by a sexual trait. Only if both these conditions are fulfilled is there a possibility for the new adaptation to be selected for and to be consolidated in later generations. The original species is likely to become less abundant in, or disappear from the sink as it lacks the necessary adaptation to exploit its resources as efficiently as the new species. The chance that this happens is highly dependent on niche pressure (JOCQUÉ, 1982), the extent to which the resources in the sink are underexploited. There is a fundamental difference in both types of speciation: in the former, often the result of geographical isolation, the complexity of the SSC does not have to change as the degree of adaptation remains similar. In the second type of speciation on the other hand, the newly acquired characters will need a parallel increase in the complexity of the SSC.

Slight changes in the extent of an SSC have often been interpreted as reinforcement; in the presence of a closely related species, SSC's would be emphasised in order to enhance the species' isolation. In the light of the present hypothesis this should rather be seen as a result of the optimal circumstances in which the species live. The fact that only one of a closely related pair of species is able to occupy a particular habitat indicates that the resources are limited. The SSC will then be less pronounced and show average diffe-

rences in a situation where resources are so abundant that closely related species are able to cohabit.

CONCLUSIONS

In contrast to the classical female choice hypotheses, mate check can explain why species are stable; it also explains why particular taxa are prone to radiate whereas others are not; mate check further explains why in some species SSC are complex, and may be so even in species living in isolation, whereas they are simple in many others.

The present hypothesis might eventually provide the answer to the old question « what comes first in a speciation event, the adaptive character that enables a new species to profit from underexploited resources or the SSC that characterises the species? ». The answer is that they both occur at the same time and that, at least a behavioural novelty has to be backed up by a SSC, otherwise it can't be consolidated.

In the present context it would seem that female choice is not a special separate mechanism accelerating radiation, but is a result of mate check.

ACKNOWLEDGEMENTS

REFERENCES

ALATALO, R.V., J. HÖGLUND & A. LUNDBERG (1991) – Lekking in the black grouse – a test of male viability. *Nature*, **352**: 155-156.

ANDERSSON, M. (1994) – *Sexual selection*. Princeton University Press. Princeton , New Jersey, 599 p.

ANDERSSON, M. & Y. IWASA (1996) – Sexual selection. *Trends in ecology and evolution*. **11**: 53-58.

ARNOLD, E.N. (1973) – Relationships of the palaearctic lizards assigned to the genera *Lacerta*, *Algyroides* and *Psammodromus* (Reptilia: Lacertidae). *Bull. Br. Mus. Nat. Hist. (Zool.)*, **25**: 291-366.

ARNQVIST, G. (1997) – The evolution of animal genitalia: distinguishing between hypotheses by single species studies. *Biol. J. Linn. Soc.*, **60**: 365-379.

ARNQVIST, G. (1998) – Comparative evidence for the evolution of genitalia by sexual selection. – *Nature*, **393**: 784-786.

BAEHR, B. & R. JOCQUÉ (1994) – Phylogeny and zoogeography of the Australian genus *Storena* (Araneae, Zodariidae). *Spixiana*, **17**: 1-12.

BAEHR, B. & R. JOCQUÉ (1996) – A revision of *Asteron,* starring male palpal morphology. *Revue Suisse Zool.* hors série: 15-28.

BOSSELAERS, J. (1998) – *Nesticus henderickxi* (Araneae, Nesticidae), a new blind troglobitic spider from Crete. *Bull. Br. Arachnol. Soc.*, **11** : 9-14.

BOSSELAERS, J. & J.-C. LEDOUX (1998) – Description of a new African genus, *Hortipes* (Araneae, Liocranidae). *Revue Arachnol.*, **12** : 147-152.

CATCHPOLE, C.K. (1987) – Bird song, sexual selection and female choice. *TREE*, **2** : 94-97.

DARWIN, C. (1859) – *The origin of species*. London, J. Murray.

DARWIN, C. (1871) – *The descent of man and selection in relation to sex*. London, J. Murray.

DE MEYER, M. (1995) – The pipunculid flies of Israel and the Sinai. *Spixiana*, **18** : 283-319.

DE MEYER, M. (1996) – Cladistic and biogeographic analyses of Hawaiian Pipunculidae (Diptera) revisited. *Cladistics*, **12** : 291-303.

DIAS, P. (1996) – Sources and sinks in population biology. *TREE*, **11** :326-330.

EBERHARD, W.G. (1985) – *Sexual selection and animal genitalia*. Harvard University Press. Cambridge, Massachussets. 231 p.

EBERHARD, W.G. (1996) – *Female control: sexual selection by cryptic female choice*. Princeton University Press, Chichester, West Sussex.

EBERHARD, W., B. HUBER B. , R. RODIGUEZ, R. BRICENO, I. SALAS & V. RODRIGUEZ (1998) – One size fits all? Relationships between the size and degree of variation in genitalia and other body parts in twenty species of insects and spiders. *Evolution*, **52** : 415-431.

ELDREDGE N. & S. GOULD (1972) – Punctuated equilibria : an alternative to phyletic gradualism. In : Ed. Schopf, T. *Models in Palaeobiology*. Freeman, Cooper & Co. San Francisco, 82-115.

FISHER, R.A. (1930) –.*The genetical theory of natural selection*. Clarendon Press, Oxford, 272p.

FRYER, G. (1991) – The evolutionary biology of African cichlid fishes. *Ann. Mus. r. Afr. Centr. Sc. Zool.*, **262** : 13-22.

GALIS, F. & J. METZ (1998) – Why are there so many cichlid species? *TREE*, **13** : 1-2.

GOULD, S. & N. ELDREDGE (1986) – Punctuated equilibrium at the third stage. *Syst. Zool.*, **35** : 145-148.

GRAFEN, A. (1990) – Sexual selection unhandicapped by the Fisher porcess. *J. theor. Biol.*, **144** : 473-516.

GRANT, P.R. (1965) – Plumage and the evolution of birds on islands. *Syst. Zool.*, **14** : 47-52.

GRISWOLD, C. (1993) – Investigations into the phylogeny of the lycosoid spiders and their kin. (Arachnida : Araneae : Lycosoidea). *Smithson. Cont. Zool.*, **539** : 1-39.

GUSTAFSSON, L. (1986) – Lifetime reproductive success and heritability: empirical support of Fisher's fundamental theorem. *Am. Nat.*, **128** : 761-764.

HEYWOOD, J. (1989) – Sexual selection by the handicap mechanism. *Evolution*, **43** : 1387-1397.

HOFFMAN, R. & K. HOWELL (1985) – A new Tanzanian genus of paradoxosomatid millipeds with medusiform gonopods (Diplopoda : Paradoxosomatidae). *Revue Zool. afr.*, **99** : 57-61.

HORNE D.J., D.L. DANIELOPOL & K. MARTENS (in press) – Reproductive behaviour. *In:* MARTENS K. (ed.) *Sex and parthenogenesis: evolutionary ecology of reproductive modes in non-marine ostracods* : 157-196. Backhuys, Leiden.

HUBER, B. (1996) – Genitalia, fluctuating asymmetry and patterns of sexual selection in *Physocyclus globosus* (Araneae, Pholcidae). *Rev. Suisse Zool.* hors série : 289-294.

HUBER B. & W. EBERHARD (1997) – Courtship, copulation and genital mechaninism in *Physocyclus globosus* (Araneae, Pholcidae). *Can. J. Zool.*, **75** : 905-918.

HURST, L.D. & A. POMIANKOWSKI (1998) – The eyes have it. *Nature*, **391** : 223-224.

JOCQUÉ, R. (1982) – Niche Pressure and the optimum exploitation hypothesis. *Biol. Jb. Dodonaea*, **50**: 168-181.

JOCQUÉ, R. (1984) – Considérations concernant l'abondance relative des araignées errantes et des araignées à toile vivant au niveau du sol. *Revue Arachnol.*, **5**: 193-204.

JOCQUÉ, R. (1990) – A revision of the afrotropical genus *Diores* (Araneae, Zodariidae). – *Annales Mus. R. Afr. Centr.* **260**: 1-81.

JOCQUÉ, R. & B. BAEHR (1992) – A revision of the Australian spider genus *Storena* (Araneae, Zodariidae). *Invert. Taxon.*, **6**: 953-1004.

JOCQUÉ, R. & L. BAERT (1996) – *Tenedos*, an early conquest of America. *Revue Suisse Zool.* hors série: 309-320.

KANESHIRO, K.Y. (1983) – Sexual selection and direction of evolution in the biosystematics of Hawaiian Drosophilidae. *Ann. Rev. Entomol.*, **28**: 161-178.

KAUFMAN, L.S., L.J. CHAPMAN & C.A. CHAPMAN (1997) – Evolution in fast forward: haplochromine fishes of the Lake Victoria region. *Endeavour*, **21**: 23-30.

KIRKPATRICK, M. (1985) – Evolution of female choice and male parental investment in polygynous species: the demise of the «sexy son». *Am. Nat.*, **125**: 788-810.

KIRKPATRICK, M. (1987) – The evolutionary forces acting on female mate preferences in polygynous animals. In: Eds. Bradbury, J.W. & M.B. Anderson *Sexual selection: Testing the alternatives*. 67–82. Wiley, Chichester.

KOCHMER, J.P. & R.H. WAGNER (1988) – Why are there so many kinds of passerine birds? Because they are small. A reply to Raikov. *Syst. Zool.*, **37**: 68-69.

LACK, D. (1968) – *Ecological adpatations for breeding birds*. Methuen. London.

LANDE, R. (1981) – Models of speciation by sexual selection on polygenic traits. *Proc. Natl. Acad. Sci. USA*, **78**: 3721-3725.

MAC ARTHUR, R.H. & E.O. WILSON (1967) – *The theory of island biogeography*. Princeton University Press, 203 pp.

MCPHAIL, J.D. (1969) – Predation and the evolution of a stickleback (*Gasterosteus*). *J. Fish. Res. Bd. Canada*, **26**: 3183-3208.

MARTENS, K. (in press) – On the origin and development of different recognition systems in the limnocytherinae (Crustacea, Ostracoda, Cytherocopina): chance or selection? *In:* HORNE, D.J. & MARTENS K. (eds.), Proc. 13 Int. Symp. Ostracodologists. *Hydrobiologia*.

MAYR, E (1963) – *Animal species and evolution*. Harvard University Press, Cambridge, Massachussets.

MØLLER, A.P. (1994) – *Sexual selection and the barn swallow*. Oxford series in Ecology and Evolution. Oxford University Press, 365 pp.

MØLLER, A. & A. POMIANKOWSKI (1993) – Fluctuating asymmetry and sexual selection. *Genetica*, **89**: 267-279.

OTTO, S.P. & Y. MICHALAKIS (1998) – The evolution of recombination in changing environments. *TREE*, **13**: 145-151.

PETRIE, M. (1992) – Peacocks with low mating success are more likely to suffer predation. *Anim. Behav.*, **43**: 173-175.

PETRIE, M. (1994) – Improved growth and survival of offspring of peacocks with more elaborate trains. *Nature*, **37**: 598-599.

RAIKOW, R.J. (1986) – Why are there so many kinds of passerine birds? *Syst. Zool.*, **35**: 255-259.

RAIKOW, R.J. (1988) – The analysis of evolutionary success. *Syst. Zool.* **37**: 76-79.

RYAN, M.J. (1990) – Sexual selection and communication in a neotropical frog, *Physalaemus pustulosus. Evolution*, **37**: 261-272.

RYAN, M.J., J.H. FOX, W. WILCZYNSKI & A.S. RAND (1990) – Sexual seletion for sensory exploitation in the frog *Physalaemus pustulosus. Nature*, **343**: 66-67.

RYAN, M.J. & A. KEDDY-HECTOR (1992) – Directional patterns of female mate choice and the role of sensory biases. *Am. Nat.* S4-S35.

SEEHAUSEN, O., J. VAN ALPHEN & F. WITTE (1997) – Cichlid fish diversity threatened by eutrophication that curbs sexual selection. *Science*, **277**: 1808-1811.

SEEHAUSEN, O. & J. VAN ALPHEN (1998) – The effect of male coloration on female mate choice in closely related Lake Victoria cichlids (*Haplochromis nyererei)* complex. *Behav. Ecol. Sociobiol.*, **42**: 1-8.

SMITH, J.N.M. (1988) – Determinants of lifetime reproductive success in the song sparrow. In : ed. T.H. CLUTTON-BROCK. *Reproductive success*, 154-172. University of Chicago Press, Chicago.

SNOEKS, J. (1991) – The use of a standard colour guide and subtle morphological difference in Lake Kivu haplochromine taxonomy. *Ann. Mus. r. Afr. Centr. Sc. Zool.*, **262**: 103-108.

SÖLI, G. (1997) – The adult morphology of Mycetophilidae (s.str.), with a tentative phylogeny of the family (Diptera, Sciaroidea). *Ent. scand. Suppl.*, **50**: 5-55.

WEISS, I. & S. HEIMER (1982) – Zwei neue *Carpathonesticus*-Arten aus Rumänien nebst betrachtungen über Kopulationsmechanismen und deren Evolution. *Reichenbachia*, **20**:167-174.

WEYGOLDT, . (1969) – *The biology of Pseudoscorpions*. Harvard University Press. Cambridge, Massachussets. 145 p.

WEYGOLDT, P. & P. HOFFMANN (1995) – Reproductive behaviour, spermatophores and female genitalia in the whip spiders *Damon diadema* (Simon, 1876), *Phrynichus* cf. *ceylonicus* (C.L. Koch, 1843) and *Euphrynichus alluaudi* (Simon, 1936)(Chelicerata : Amblypygi). *Zool. Anz.* 234: 1-18.

WIENS, J.A. (1979) – On competition and variable environments. *Am. Sci.*, **65**: 590-597.

WILKINSON, G.S., D.C. PRESGRAVES & L. CRYMES (1998) – Male eye span in stalk-eyed flies indicates genetic quality by meiotic drive suppression. *Nature*, **391**: 276-279.

WILLIAMS, G.C. (1975) – *Sex and evolution*. Princeton University Press., Princeton, New Jersey.

WILLIAMS, G.C. (1992) – *Natural selection: Domains, levels and challenges*. Oxford University Press. Oxford.

ZAHAVI, A. (1975) - Mate selection, - a selection for a handicap. *J. theor. Biol.*, **53**: 205-214.

ZAHAVI, A. (1987) - The theory of signal selection and some of its implications. In : Ed. DELFINA, V.P. *International symposium of Biological Evolution*. Adriatic editrice, Bari. 305-327.

ZEYL, C. & G. BELL (1997) – The advantage of sex in evolving yeast populations. *Nature*, **388**: 465-467.

GENETIC COMPARISON
OF TWO COLOUR MORPHS OF *PHYLLOTRETA TETRASTIGMA*
(COLEOPTERA : CHRISOMELIDAE, ALTICINAE)

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Abstract. In the flea beetle species *Phyllotreta tetrastigma* two colour morphs (2B and 4S) exist in western Europe. We test whether these forms are genetically differentiated using two techniques : Polyacrylamide gel electrophoresis (PAGE) of allozyme loci and Isoelectric Focusing (IEF) of general proteins. PAGE reveals that both forms are not genetically isolated and IEF did not show any differences in banding patterns between them. Both forms are considered to interbreed and to belong to the same gene pool.

Key words : color forms, *Phyllotreta*, Chrysomelidae, genetics, allozymes, Isoelectric Focusing.

INTRODUCTION

Colour polymorphism is known in many different groups of animals (*e.g.* BOOTH, 1990 ; CORDERO, 1990 ; BACKELJAU *et al.* 1992 ; HOLLOWAY, 1993). Variation in colour can be due to host plant choice, season, temperature during development and/or can be genetically determined. Undoubtedly, the best studied cases are of species of Lepidoptera (*e.g.* COOK *et al.*, 1990 ; KINGSOLVER & WIERNASZ, 1991 ; SMITH *et al.*, 1993 ; PAULSEN, 1994) and these have aroused much interest concerning the adaptive value, heritability and phenotypic plasticity of their different colorations.

Although more limited in number, several genetic studies on beetle species considered colour polymorphism. LIEBHERR (1983) showed that in the ground beetle *Agonum decorum* Say, 1823 the red-green colour polymorphism and hirsute-glabrous setational polymorphism are determined by unlinked, autosomal diallelic loci. HANTULA *et al.* (1987) failed to detect genetic differences associated with colour polymorphism in the weevil *Diaprepes abbreviatus* (L., 1764).

In chrysomelid beetles the amount of colour variation is often very large, but the number of studies on the phenomenon remains rather limited. In chrysomelid systematics colour characteristics have been used to distinguish between different species (BROWN,

1956 ; MOHR, 1966 ; DOGUET, 1986, 1994), but in many cases (and often in the very same studies), the systematic value of colour characteristics is questioned (BROWN, 1956 ; DOGUET, 1986) as a considerable amount of variation in colour pattern is found within a single species, and sometimes even within a single population. In some cases ecological differences are detected which can be helpfull in making systematic decisions (BROWN, 1956), but in most cases they do not allow authors to conclude whether different colour forms are genetically isolated, and thus represent biological species, or not. In several instances a more profound study revealed that systematic differences based on coloration were incorrect.

· Probably the first study on the genetics of colour polymorphism in chrysomelids was the work of THOMAS (1964) on the cassid beetles of the genus *Aspidomorpha* Hope, 1840. He discovered that three former species (*A. adhaerens* (Weber,1801), *A. testudinaria* (Montrouzier, 1855) and *A. phyllis* (Boheman, 1862)) were in fact colour morphs of a single species. FUJIYAMA & ARIMOTO (1988) found random mating between two colour forms of *Chrysolina aurichalcea* (Mannerheim). VASCONCELLOS-NETO (1988) studied the genetics of *Chelymorpha cribraria* (Fabricius, 1775) and found that the elytral colour is expressed by three different alleles of a single gene, whereas pronotum colour is a quantitative character under polygenic control. BOITEAU *et al.* (1994) studied the genetics of a beige elytral mutant of the Colorado potato beetle *Leptinotarsa decemlineata* Say, 1824 and found that inheritance was controlled by two dominant genes. BOITEAU (1994) discusses the genetics of several mutations (white body, pearleye, black body and beige elytra) of *L. decemlineata* and discovered that the beige mutant has lower fitness. LU & LOGAN (1994) showed that larval colour variation in *L. decemlineata* is controlled by two epistatic loci. VERDYCK *et al.* (1996) did not find genetic isolation between two colour forms of *Phyllotreta cruciferae* (Goeze, 1777).

Here we study *Phyllotreta tetrastigma* (Comolli, 1837), one of the larger species within the genus *Phyllotreta* (Coleoptera : Chrysomelidae), widely distributed throughout Europe and Western Asia. The species is closely related to *P. dilatata* Thomson, 1866 and *P. flexuosa* (Illiger, 1794), from which it is distinguished by minor differences in the elytral colour pattern (DOGUET, 1986, 1994). *P. tetrastigma* is a monophagous species (NIELSEN, 1978b) feeding only on *Cardamine sp.* It is almost exclusively found on large bittercress, *Cardamine amara* (NIELSEN, 1978a ; NIELSEN, 1978b), though we found one population feeding on *Cardamine flexuosa*. This species is monophagous in the field, but in laboratory conditions it will also feed on other cruciferous plants (NIELSEN, 1978b).

Within *P. tetrastigma*, two types of elytral colour pattern can be distinguished. Type 2B has a yellow band (which is narrowed in the middle) on each elytron. Type 4S has two yellow spots on each elytron. Variation within each type is considerable and few specimens are intermediate. In most places both forms can be found, which means that they are sympatric, even the series of syntypes contains specimens of both forms (VERDYCK *et al.*, 1995). VERDYCK *et al.* (in press) did not find morphological differences between them. This study aims to test for genetic isolation between the two colour forms of *P. tetrastigma*, and to examine several aspects of population genetics in the species.

MATERIAL AND METHODS

Populations studied

Six populations of *P. tetrastigma,* in which both forms were present were sampled in Western Europe in the period 1990 to 1994 (Table 1). All animals were collected from the leaves of their host plant using an aspirator. In all cases the host plant was *Cardamine amara*, except in the Udenhout (NL) population where the animals fed on *Cardamine flexuosa*. All habitats sampled were characterised as wet and shady places in woods. Animals were transported alive to the laboratory or immediately frozen in liquid nitrogen. They were stored at -80°C until sample preparation.

TABLE 1

Populations and numbers of P. tetrastigma *studied,
numeric contribution of both forms (4S-2B) in each population*

Locality	Country	Lon, Lat	No of specimens	4S	2B	% 4S	% 2B
Celles sur Plaine	France	48/57'N, 6/57'E	127	20	107	15.7	84.3
Chimay	Belgium	50/3'N, 4/19'E	119	14	105	11.8	88.2
Geisenfeld	Germany	48/41'N, 11/37'E	84	6	78	7.1	92.9
Stenholts Vang	Denmark	55/57'N, 12/21'E	64	14	50	21.9	78.1
Udenhout	Netherlands	51/37'N, 5/9'E	77	13	64	16.9	83.1
Zoersel	Belgium	51/16'N, 4/42'E	220	72	148	32.7	67.3
Total			691	139	552	20.1	79.9

Electrophoretic analysis

For sample preparation the abdomens of the animals were removed and single abdomens were homogenized in 25µl sucrose solution (20% w/v). Crude homogenates were centrifuged at 4°C for 45 minutes at 15,000 rpm (27.200 g) and were stored at -80°C until electrophoresis.

Nine enzyme systems coding for ten different loci were screened: aconitase (*ACON*, E.C. 4.2.1.3), α-amylase (*AMY*, E.C. 3.2.1.1), α-glycerophosphate dehydrogenase (*GPD*, E.C. 1.1.1.8), aspartate aminotransferase (*AAT*, E.C. 2.6.1.1), isocitric dehydrogenase *(ICD*, E.C. 1.1.1.42), malate dehydrogenase (*MDH,* E.C.1.1.1.37), mannose phosphate isomerase (*MP-1* and *MP-2*, E.C. 5.3.1.8 [2 loci]), peptidase (Leu-Ala) (*PEP*, E.C. 3.4.-.-) and phosphoglucomutase (*PGM*, E.C. 5.4.2.2.). Staining recipes were adapted from HARRIS & HOPKINSON (1976). The buffer systems used were a Tris/Citric Acid (TC) system pH 8.0 (0.1M) [for *ACON, AMY, GPD, ICD, MP-1, MP-2* and *PGM*] and a Tris/Boric Acid/EDTA (TBE) system pH 8.9 (0.1M) [for *AAT, MDH* and *PEP*]. For each sample 5µl of supernatant was applied to 6% polyacrylamide gels. Vertical polyacrylamide gel electrophoresis (PAGE) was performed with Hoefer Mighty Small System II, running gels for 15 minutes at 25 Volts, then 15 minutes at 50 Volts and finally for 1 or 3 hours at 150 Volts (for TC and

TBE buffer respectively). Alleles were designated alphabetically according to decreasing mobility, the fastest allele (the most anodal one) being A.

Hardy-Weinberg equilibrium

The six geographic populations (4S and 2B combined) were tested for deviations from Hardy-Weinberg (HW) equilibrium using exact probabilities (SWOFFORD & SELANDER, 1989), (corrected for multiple comparisons using sequentially rejective Bonferroni test). The same was done for the 4S and 2B form within each locality.

Population differentiation

Hierarchical F-statistics (WRIGHT, 1965, 1978) were used to analyse genetic differentiation at two different levels. In our hierarchy F refers to form, L to locality and T to total. In this study we have six populations, and within each population we have two forms: 4S and 2B. In this way differentiation among forms within localities is described by F_{FL}, and so on. We also performed contingency table analyses of heterogeneity among forms (for each population) and among populations, and calculated fixation indices F and coefficients of heterozygote deviation D.

Genetic distances, Clustering
and Multivariate Analysis

There has been much discussion on which clustering method is the best, and in his overview BUTH (1984) concluded that for closely related species there are difficulties in obtaining a correct topology with most methods. Here the two most predominantly used genetic distances, (NEI (1978) unbiased genetic distances and Modified Rogers distances (WRIGHT, 1978)) were calculated between populations. Both were used for the construction of UPGMA dendrograms, but only Rogers distance could be used for construction of a Wagner tree with midpoint rooting. A multivariate analysis of the allele frequencies was performed using the correspondence analysis (CA option) of the program CANOCO (V3.2) (TER BRAAK, 1988), and the population scores on the two first canonical axes are plotted.

Isoelectric Focusing

A second evaluation of genetic differentiation was performed using Isoelectric Focusing (IEF) of general proteins. This was done with PhastSystem from Pharmacia LKB. Using a 8/1 sample applicator, 1:1 of sample was applied to an IEF gel with pH range 4-6.5. Samples of both forms were chosen randomly from specimens of the six populations. The program running conditions are as in VERDYCK et al. (1992). After a run of 500 Vh the proteins have moved to their isoelectric point and are visualized by means of silver staining according to the PhastSystem IEF silver staining program performed with PhastSystem developing unit. Gels were air dried and maintained in standard 4x4 cm slides for storage and further manipulation.

Gels were projected on a slide viewer for visual interpretation. As only adjacent lanes were compared (the same lane not being used twice), each gel (8 lanes) gave 4 comparisons. Counts were made for the number of bands visible in both lanes and for the number of bands unique for lane one and lane two respectively. Lanes of bad quality were excluded from analysis and parts of lanes that were not clearly interpretable were not used.

The mean number of bands for the two forms was compared using a Mann-Whitney U test. We calculated three different similarity measures. The similarity S_F as defined by FERGUSON (1980) and used in similar studies of BACKELJAU (1985) and VERDYCK et al. (1992, 1996) is defined as the number of bands of common mobility divided by the maximum number of bands for an individual. The Jaccard (S_J) and Dice (S_D) indices, as defined in SNEATH & SOKAL (1973), take into account both the specimens sampled (thus avoiding strong influences of individuals with an extremely high number of bands). Bands in common in both individuals are given more weight in S_D.

Three groups of similarities (between two 4S forms (4S4S), between two 2B forms (2B2B) and between 4S and 2B forms (4S2B) are calculated (4S4S and 2B2B = intraform similarities, 4S2B = interform similarities)). Fifteen comparisons were used for each group. The three different similarities were compared using ANOVA. To avoid interdependance of the comparisons, individuals were never used twice.

RESULTS

Populations studied

In total we screened 691 animals (139 of the 4S form and 552 of the 2B form) (Table 1). In all six populations studied the 4S form is less abundant compared to the 2B form. The proportion of the 4S form varies from 7.1 to 32.7 % (mean ± st.dev. : $17.7 ± 8.9$), the proportion of the 2B form varies from 67.3 to 92.9 % (mean ± st.dev. : $81.5 ± 10.6$). In many studies coloration types are known to vary geographically (*e.g.* ABBAS, 1988; BURKE, 1989; SILFVERBERG, 1991, 1994). Here we always find dominance of the same form. Of course a study on geographic variation should include many more populations. As *P. tetrastigma* has only one generation each year, and the adults are only active during a short period in which their host plants are abundant, seasonal variation can be excluded.

Electrophoretic analysis

All loci screened, with in total 25 alleles, are polymorphic (Table 2). Three loci (*AAT, MP-2* and *PGM*) are polymorphic at all localities, *aco* is polymorphic at four, *MP-1* and *AMY* at three and *ICD* at two localities. The remaining loci (*MDH, PEP* and *GPD*) are only polymorphic in one locality. None of the populations is polymorphic for all 10 loci. The highest percentage of loci polymorphic is found in Chimay (80%), the lowest in Celles sur Plaine and Stenholts Vang (both 40 %). An example of an allozyme profile is shown in Fig. 1.

No diagnostic loci for the colour forms were found. Five alleles (*MDH*-B, *PEP*-B, *GPD*-B, *MP-1*-A and *MP-2*-A) were only found in the 2B form, one allele (*AAT*-D) was

only found in the 4S form. But all of these alleles had very low frequencies (<0.005, except for *MP-1*-A with 0.012), and can be considered rare alleles.

TABLE 2

Allele frequencies for 10 allozyme loci in six populations of P. tetrastigma

Locus	Allele	Celles sur Plaine	Chimay	Geisenfeld	Stenholts Vang	Udenhout	Zoersel
AAT	n	61	46	40	39	40	81
	A	0.516	0.467	0.563	0.397	0.675	0.543
	B	0.451	0.500	0.425	0.577	0.287	0.457
	C	0.025	0.033	0.013	0.026	0.038	0.000
	D	0.008	0.000	0.000	0.000	0.000	0.000
MDH	n	63	46	40	39	40	88
	A	1.000	1.000	1.000	1.000	1.000	0.994
	B	0.000	0.000	0.000	0.000	0.000	0.006
PEP	n	47	41	40	24	14	56
	A	1.000	0.988	1.000	1.000	1.000	1.000
	B	0.000	0.012	0.000	0.000	0.000	0.000
ACON	n	41	50	40	38	44	90
	A	0.098	0.110	0.025	0.000	0.000	0.006
	B	0.902	0.890	0.975	1.000	1.000	0.994
GPD	n	46	46	33	34	23	46
	A	1.000	1.000	1.000	1.000	1.000	0.989
	B	0.000	0.000	0.000	0.000	0.000	0.011
MP-1	n	52	55	35	26	35	54
	A	0.000	0.036	0.014	0.000	0.014	0.000
	B	1.000	0.964	0.986	1.000	0.986	1.000
MP-2	n	45	51	43	25	27	48
	A	0.000	0.010	0.000	0.000	0.000	0.000
	B	0.878	0.990	0.965	0.840	0.889	0.865
	C	0.122	0.000	0.035	0.160	0.111	0.135
ICD	n	50	48	39	24	46	100
	A	1.000	0990	1.000	0.979	1.000	1.000
	B	0.000	0.010	0.000	0.021	0.000	0.000
AMY	n	44	55	36	11	41	83
	A	1.000	0.991	1.000	1.000	0.976	0.988
	B	0.000	0.009	0.000	0.000	0.024	0.012
PGM	n	59	49	36	18	37	93
	A	0.025	0.051	0.056	0.000	0.176	0.059
	B	0.593	0.724	0.625	0.694	0.257	0.667
	C	0.356	0.214	0.278	0.306	0.446	0.247
	D	0.025	0.010	0.042	0.000	0.122	0.027
	H_{exp}	0.145	0.132	0.119	0.127	0.144	0.130
	H_{obs}	0.121	0.114	0.103	0.124	0.104	0.099

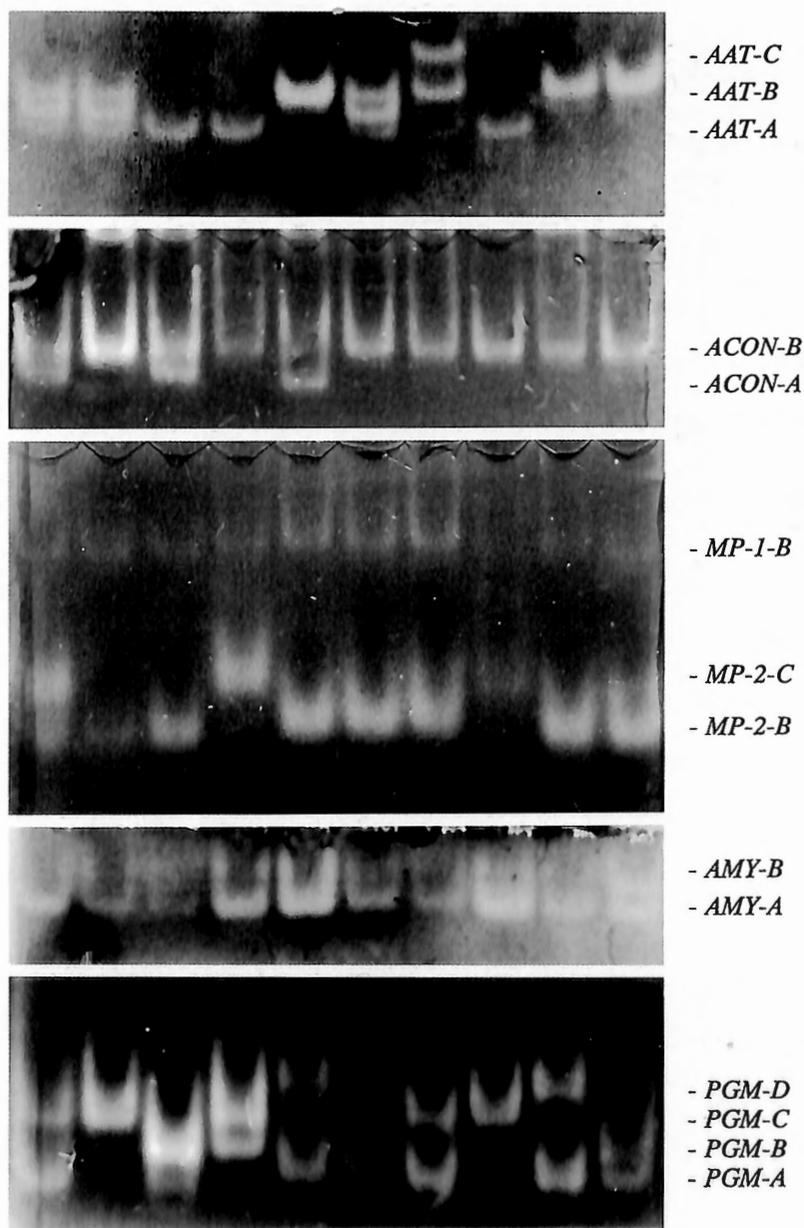H_{exp} : expected heterozygosity assuming Hardy-Weinberg equilibrium ; H_{obs} : observed heterozygosity

Fig. 1. – Example of allelic variation in the most variable loci : from top to bottom :
AAT, ACON, MP-1 and MP-2, AMY, PGM (alleles are indicated)

Hardy-Weinberg equilibrium

The analysis for the six localities (the two forms mixed) shows only two deviations from Hardy-Weinberg equilibrium: *MP-2* for Celles sur Plaine and Zoersel (exact probabilities, sequential Bonferroni corrected, $p < 0.05$) (Table 3). Both significant deviations from Hardy-Weinberg equilibrium are caused by heterozygote deficiencies (Table 3).

TABLE 3

Significance test using exact probabilities (sequential Bonferroni corrected), fixation index F and coefficient for heterozygote deficiency D of allozyme loci

Locus	Celles sur Plaine	Chimay	Geisenfeld	Stenholts Vang	Udenhout	Zoersel
AAT	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	F=-0.208	F=0.180	F=0.055	F=-0.210	F=0.022	F=0.055
	D=0.198	D=-0.189	D=-0.167	D=0.195	D=-0.035	D=-0.061
MDH	-	-	-	-	-	n.s.
	-	-	-	-	-	F=-0.006
	-	-	-	-	-	D=0.000
PEP	-	n.s.	-	-	-	-
	-	F=-0.012	-	-	-	-
	-	D=0.000	-	-	-	-
ACON	n.s.	n.s.	n.s.	-	-	n.s.
	F=0.446	F=0.285	F=1.000	-	-	F=-0.006
	D=-0.453	D=-0.292	D=-1.000	-	-	D=0.000
GPD	-	-	-	-	-	n.s.
	-	-	-	-	-	F=-0.011
	-	-	-	-	-	D=0.000
MP-1	-	n.s.	n.s.	-	n.s.	-
	-	F=-0.038	F=-0.014	-	F=-0.014	-
	-	D=0.028	D= 0.000	-	D=0.000	-
MP-2	p<0.05	n.s.	n.s.	n.s.	n.s.	p<0.05
	F=0.689	F=-0.010	F=0.655	F=0.702	F=0.625	F=0.733
	D=-0.693	D=0.000	D=-0.659	D=-0.708	D=-0.632	D=-0.736
ICD	-	n.s.	-	n.s.	-	-
	-	F=-0.011	-	F=-0.021	-	-
	-	D=0.000	-	D=0.000	-	-
AMY	-	n.s.	-	-	n.s.	n.s.
	-	F=-0.009	-	-	F=1.000	F=-0.012
	-	D=0.000	-	-	D=-1.000	D=0.006
PGM	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	F=0.218	F=0.043	F=0.052	F=-0.178	F=0.294	F=0.210
	D=-0.225	D=-0.053	D=-0.065	D=0.145	D=-0.304	D=-0.215

The analysis for all localities divided in two forms reveals similar results. We find significant deviations from Hardy-Weinberg equilibrium for *MP-2* at the same localities. Both deviations are found in the 2B form (in Celles sur Plaine the 4S form is not polymorphic).

Population differentiation

Table 4 shows the variance components and hierarchical F-statistics combined across loci for the different levels. For this multilocus estimate the variance was smaller among forms within localities than among localities within the total population, and F_{FL} is smaller than F_{LT}.

TABLE 4

Variance components and F-statistics combined across allozyme loci

X	Y	variance component	F_{XY}
form (F)	locality (L)	0.01410	0.011
form (F)	total (T)	0.03295	0.026
locality (L)	total (T)	0.01885	0.015

Contingency chi-square analysis of allele frequency differences (sequential Bonferroni corrected) between populations do not show significant heterogeneity in any of the localities (not for any locus, nor for all loci combined) between both forms (4S-2B) (p=0.05).

Chi-square analysis (for both forms combined) gives significant differentiation between localities at 5 loci *(ACON, MP-2* and *PGM)* (p<0.05). Three out of 7 other loci *(MDH, PEP* and *GPD)* are only polymorphic in one locality (table not shown).

Genetic distances, Clustering
and Multivariate Analysis

NEI (1978) unbiased genetic distance and Modified Rogers distance (WRIGHT, 1978) were calculated (table 5). The first one varied between 0.000 and 0.024 (mean = 0.007), the second one between 0.036 and 0.148 (mean = 0.078). Three dendrograms were constructed. The population of Udenhout always branched off first, while the position of the other populations changed in the different dendrograms. Only the Wagner tree based on Rogers modified distance is shown in Fig. 2.

TABLE 5

Matrix of genetic distances of allozyme data: above diagonal: NEI (1978) unbiased genetic distance, below diagonal Rogers modified distance (WRIGHT, 1978)

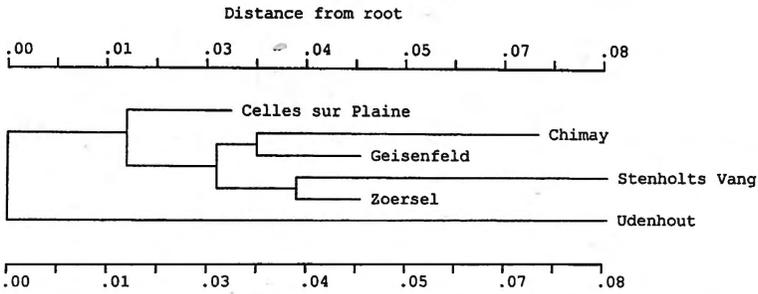| Population	1.	2.	3.	4.	5.	6.
1. Celles sur Plaine	-	0.003	0.000	0.002	0.011	0.001
2. Chimay	0.061	-	0.001	0.003	0.024	0.003
3. Geisenfeld	0.043	0.049	-	0.003	0.011	0.000
4. Stenholts Vang	0.058	0.070	0.069	-	0.022	0.001
5. Udenhout	0.106	0.148	0.107	0.146	-	0.015
6. Zoersel	0.043	0.060	0.036	0.049	0.118	-

Fig. 2. – Wagner tree based on Modified Rogers distance (WRIGHT, 1978),
Cophenetic correlation = .981, rooted at midpoint of longest path

In the correspondence analysis, the cumulative percentage of the variance explained by the canonical axes is 52.7 for CA1 and 79.1 for CA2. The populations scores plotted for CA1 and CA2 (Fig. 3) show a clear separation of the Udenhout population from the other populations according to the first axis. This axis is strongly correlated with the allele frequencies of *PGM-B, PGM-D, AAT-B, PGM-A, GOT-A* and *PGM-C*. According to the second axis the Chimay population is more or less clearly separated from the other populations, although differences here are less marked. This axis is strongly correlated with the alleles *MP2-B, MP2-C, MP1-A* and *MP1-B*.
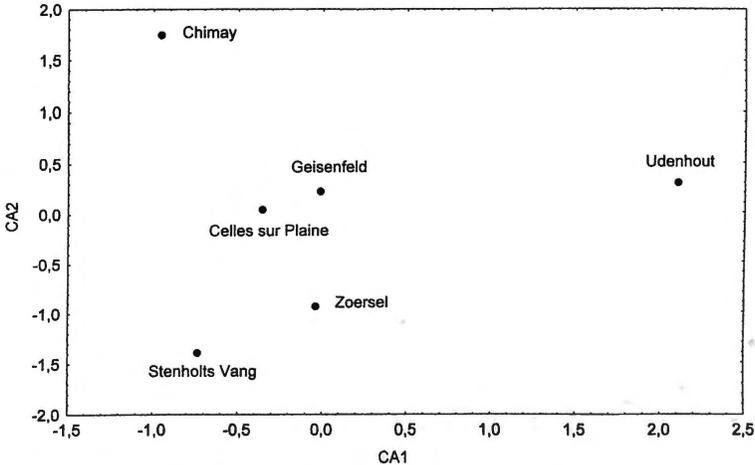
Fig. 3. – Biplot of the population scores on
the first two canonical axes of the CA analysis

Isoelectric Focusing

An example of a gel is shown in Fig. 4. The average number of bands for both forms was calculated (4S: mean ± st. dev.: 47.69 ± 6.06; 2B: mean ± st. dev.: 45.13 ± 6.02).

Between both forms no significant difference in the number of bands was detected (Mann-Whitney U test; $p < 0.05$).
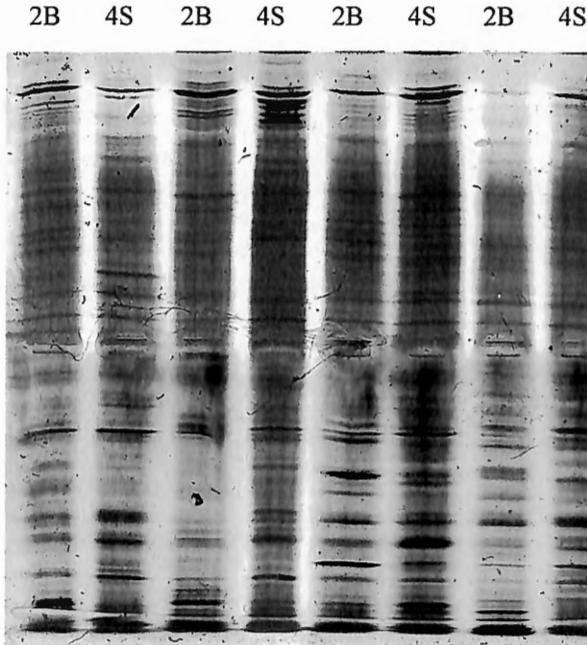
2B   4S   2B   4S   2B   4S   2B   4S

Fig. 4. – IEF pattern: forms from left to right: 2B,4S,2B,4S,2B,4S,2B,4S

Similarities S_F, S_J and S_D are given in table 6. Differences between the three types of comparisons (DD, DL and LL) were not significant (ANOVA, df = 2,42; p>0.1).

TABLE 6

Basic statistical data on IEF protein polymorphisms for similarities S_F S_J S_D for the three types of comparisons

comp.	simil.	Mean	st. dev.	# comparisons
4S4S	S_F	0.879	0.030	15
	S_J	0.822	0.050	15
	S_D	0.902	0.030	15
4S2B	S_F	0.870	0.037	15
	S_J	0.806	0.054	15
	S_D	0.892	0.033	15
2B2B	S_F	0.871	0.030	15
	S_J	0.818	0.047	15
	S_D	0.899	0.028	15

DISCUSSION

P. tetrastigma is a genetically very variable species showing high heterozygosity values and being polymorphic at all ten loci studied. VERDYCK *et al.* (1996) studied another *Phyllotreta* species (*P. cruciferae*) and found that it was only variable at five of these loci (*AAT, MDH, GPD, AMY* and *PGM*) of which only the *AAT* locus showed variation in all populations.

Genetic population structuring has been studied in several other chrysomelid species and KNOLL *et al.* (1996) give an overview of several studies with special emphasis on Alpine populations. In hierarchical studies F_{ST} values have been compared at different micro-and macrogeographic scales (between trees, localities a few kilometers apart and localities sometimes 500 km apart). In *Plagiodera versicolora* F_{ST} values between 0.006 and 0.098 were found between trees within localities (MCCAULEY *et al.*, 1988), while in *Chrysomela aeneicollis* , large F_{ST} values were found (for several loci) at the same level (RANK, 1992). Our F_{FL} value of 0.011 fits in this spectrum. Between localities F_{LT} values varied from 0.003 to 0.057 in *P. versicolora* (MCCAULEY *et al.*, 1988), from 0.010 to 0.135 in *Chrysomela aeneicollis* (RANK 1992), from 0.011 and 0.053 in *Phratora vitellinae* (Linnaeus, 1758) and from 0.066 and 0.094 in *Oreina cacaliae* (Schrank, 1785) (KNOLL *et al.,* 1996). The F_{LT} value of 0.015 for *P. tetrastigma* is (although not exceptional) rather low for populations at such geographic scale. Apparently there is an important amount of gene flow between these geographically sometimes very distant populations, leading to little genetic differentiation at a large geographic scale. Although the resolving power of band sharing with IEF is limited and the allozymes tested provide only a limited sample of the genome, we suspect that important genetic differentiation would have been detected using these techniques. A promising future strategy to further explore this question would be assessment of variability in microsatellite DNA. In that case it would be interesting to obtain more populations from a large geographic range.

The clear separation of the Udenhout population in the Wagner tree is possibly due to the fact that this was the only population feeding on *Cardamine flexuosa*. This result indicates that the formation of host plant races can lead to genetic separation and speciation, an evolutionary pathway finding support in several other insect groups. The best studied case is probably that of the sibling species in the *Rhagoletis* fruit flies (FEDER *et al.*, 1988, 1989, 1990a, 1990b; MCPHERON *et al.*, 1988; BERLOCHER *et al.*, 1993). GOYER *et al.* (1995) also demonstrated host-associated genetic differentiation in the fruittree leafroller *Archips argyrospila*, suggesting formation of sibling species by means of different hosts and GULDEMOND (1990a, 1990b) discussed host plant shift, host race formation and speciation in the aphid genus *Cryptomyzus*.

The IEF results demonstrated that both forms do not differ significantly in the number of bands, and inter- and intraform similarities do not show any significant differences, indicating lack of general protein pattern differences in both forms.

VERDYCK *et al.* (1996) did not find evidence for genetic isolation between the colour forms in *P. cruciferae*. For *P. tetrastigma* results of allozymes and IEF lead to a similar conclusion. As neither allozyme nor IEF results indicate genetic differentiation between the 4S and 2B form of *P. tetrastigma,* both forms are considered interbreeding and belong-

ing to the same populations. Colour differences in *P. tetrastigma* are without systematic value, and again confirm the conclusion of VERDYCK *et al.* (1996) that a profound study of colour variation in chrysomelid species is necessary before systematic conclusions can be drawn.

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REFERENCES

ABBAS, I., K. NAKAMURA, H. KATAKURA & H. SASAJI (1988) – Geographical variation of elytral spot patterns in the phytophagous ladybird, *Epilachna vigintioctopunctata* (Coleoptera : Coccinellidae) in the province of Sumatra Barat, Indonesia. *Res. Popul. Ecol.,* **30** : 43-56.

BACKELJAU, T. (1985) – Estimation of genic similarity within and between *Arion hortensis s.l.* and *A. intermedius* by means of isoelectric focused esterase patterns in hepatopancreas homogenates (Mollusca, Pulmonata : Arionidae). *Z. zool. Syst. Evolut.-forsch.,* **23** (1) : 38-49.

BACKELJAU, T., C.P. DE BRITO, R.M. TRISTAO DA CUNHA & A.M. FRIAS MARTINS (1992) – Colour polymorphism and genetic strains in *Arion intermedius* from Flores, Azores (Mollusca : Pulmonata). *Biol. J. Linn. Soc.,* **46** : 131-143.

BERLOCHER, S.H., B.A. MCPHERON, J.L. FEDER & G.L. BUSH (1993) – Genetic differentiation at allozyme loci in the *Rhagoletis pomonella* (Diptera : Tephritidae) species complex. *Ann. ent. Soc. Am.,* **86** (6) : 716-727.

BOITEAU, G., G.C.C.TAI & M.E. DREW (1994) – Genetics and biological fitness of a beige elytral mutant of the Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera : Chrysomelidae). *Can. J. Zool.,* **72** : 312-318.

BOITEAU, G. (1994) – Genetics of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say). In : *Novel aspects of the Biology of Chrysomelidae.* JOLIVET, P.H., COX M.L. & E. PETITPIERRE, E. (Eds.). Kluwer Academic Publishers, Dordrecht : 231-236.

BOOTH, C.L. (1990) – Evolutionary significance of ontogenetic colour change in animals. *Biol. J. Linn. Soc.,* **40** : 125-163.

BROWN, W.J. (1956) – Sibling species in the Chrysomelidae. In : *Proceedings Tenth International Congress of Entomology.* E.C. BECKER (Ed). Mortimer Limited, Ottawa, Canada : 103-109.

BURKE, D.P.T. (1989) – Variation in body colour in western Irish populations of *Cepaea nemoralis* (L.). *Biol. J. Linn. Soc.,* **36** : 55-63.

BUTH, D.G. (1984) – The application of electrophoretic data in systematic studies. *Ann. Rev. Ecol. Syst.,* **15** : 501-522.

COOK, L.M., K.D. RIGBY & M.R.D. SEAWARD (1990) – Melanic moths and changes in epiphytic vegetation in north-west England and north Wales. *Biol. J. Linn. Soc.,* **39** (4) : 343-354.

CORDERO, A. (1990) – The inheritance of female polymorphism in the damselfly *Ischnura graellsi* (Rambur) (Odonata : Coenagrionidae). *Heredity,* **64** : 341-346.

DOGUET, S. (1986) – Mise B jour du catalogue des Alticinae de la faune de France : le genre *Phyllotreta* Chevrolat (Col. Chrysomelidae). *l'Entomologiste,* **42** (3) : 145-149.

DOGUET, S. (1994) – Coléoptéres Chrysomelidae volume 2 : Alticinae. *Faune de France,* Fédération Française des Sociétés de Sciences Naturelles, Paris, France. **80** : 694 pp.

FEDER, J.L., C.A. CHILCOTE & G.L. BUSH (1988) – Genetic differentiation between sympatric host races of *Rhagoletis pomonella. Nature,* **336** : 61-64.

FEDER, J.L., C.A. CHILCOTE & G.L. BUSH (1989) – Are the apple maggot (*Rhagoletis pomonella*) and the blueberry maggot (*R. mendax*) distinct species? Implications for sympatric speciation. *Entomol. exp. appl.,* **51** : 113-123.

FEDER, J.L., C.A. CHILCOTE & G.L. BUSH (1990a) – The geographic pattern of genetic differentiation between host-associated populations of *Rhagoletis pomonella* (Diptera : Tephritidae) in the eastern United States. *Evolution,* **44** : 570-594.

FEDER, J.L., C.A. CHILCOTE & G.L. BUSH (1990b) – Regional, local, and microgeographic allele frequency variation between apple and hawthorn populations of *Rhagoletis pomonella* in Western Michigan. *Evolution,* **44** : 596-608.

FERGUSON, A. (1980) – Biochemical systematics and evolution. Blackie & Son., Glasgow.

FUJIYAMA, S. & K. ARIMOTO (1988) – Genetics of the two colour forms of *Chrysolina aurichalcea* (Mannerheim) (Coleoptera : Chrysomelidae) and their gene frequencies in two mountainous areas of central Honshu, Japan. In : *Biology of Chrysomelidae.* P. JOLIVET, E. PETITPIERRE & T.H. HSIAO (Eds.). Kluwer Academic Publishers, Dordrecht : 205-215.

GOYER, R.A., T.D. PAINE, D.P. PASHLEY, G.J. LENHARD, J.R. MEEKER & C.C. HANLON (1995) – Geographic and host-associated differentiation in the fruittree leafroller (Lepidoptera : Tortricidae). *Ann. ent. Soc. Am.,* **88** (4) : 391-396.

GULDEMOND, J.A. (1990a) – Host plant shift, host race formation and speciation in *Cryptomyzus* (Homoptera, Aphididae). *Acta Phytopathol. Ent. Hungarica,* **25** : 89-96.

GULDEMOND, J.A. (1990b) – Evolutionary genetics of the aphid *Cryptomyzus*, with a preliminary analysis of the inheritance of host plant preference, reproductive performance and host-alteration. *Entomol. exp. appl.,* **57** : 65-76.

HANTULA, J., A. SAURA, J. LOKKI & N. VIRKKI (1987) – Genic and color polymorphism in Puerto Rican phyllobiine weevils *Diaprepes abbreviatus* (L.) and *Compsus maricao* Wolcott. *J. Agric. Univ. Puerto Rico,* **4** : 391-397.

HARRIS, H. & D.A. HOPKINSON (1976) – Handbook of enzyme electrophoresis in human genetics. North Holland publishing Co., Amsterdam.

HOLLOWAY, G.J. (1993) – Phenotypic variation in colour pattern and seasonal plasticity in *Eristalis* hoverflies (Diptera : Syrphidae). *Ecol. Entomol.,* **18** : 209-217.

KINGSOLVER, J.G. & D.C. WIERNASZ (1991) – Development, function, and the quantitative genetics of wing melanin pattern in *Pieris* butterflies. *Evolution,* **45** (6) : 1480-1492.

KNOLL, S., M. ROWELL-RAHIER, P. MARDULYN & J.M. PASTEELS (1996) Spatial genetic structure of leaf beetle species with special emphasis on Alpine populations. In : *Chrysomelidae Biology, vol. 1 : The classification, phylogeny and genetics.* P.H.A. JOLIVET & M.L. COX (Eds.). SPB Academic Publishing, Amsterdam, The Netherlands : 379-388.

LIEBHERR, J.K. (1983) – Genetic basis for polymorphism in the ground beetle, *Agonum decorum* (Say) (Coleoptera : Carabidae). *Ann. ent. Soc. Am.,* **76** (3) : 349-358.

LU, W. & P. LOGAN (1994) – Inheritance of larval body color in *Leptinotarsa decemlineata* (Coleoptera : Chrysomelidae). *Ann. ent. Soc. Am.* **87**(4): 454-459.

McCAULEY, D.E., M.J. WADE, F.J. REDEN & M. WOHLTMAN (1988) – Spatial and temporal variation in group relatedness : evidence from the important willow leaf beetle. *Evolution,* **42** : 184-192.

McPHERON, B.A., D.C. SMITH & S.H. BERLOCHER (1988). – Genetic differences between host races of the apple magot fly. *Nature,* **336** : 64-66.

MOHR, K.H. (1966) – Chrysomelidae. In : *Die Käfer Mitteleuropas.* 9, H. FREUDE, K.W. HARDE & G.A. LOHSE (Eds.). Goecke & Evers, Krefeld : 95-297.

NEI, M. (1978) – Estimation of average heteroygosity and genetic distance from a small number of individuals. *Genetics,* **89** : 583-590.

NIELSEN, J.K. (1978a) – Host plant discrimination within cruciferae : feeding responses of four leaf beetles (Coleoptera : Chrysomelidae) to glucosinolates, cucurbitans and cardenolides. *Entomol. exp. appl.,* **24** : 41-54.

NIELSEN, J.K. (1978b) – Host plant selection of monophagous and oligophagous flea beetles feeding on crucifers. *Entomol. exp. appl.,* **24** : 362-369.

PAULSEN, S.M. (1994) – Quantitative genetics of butterfly wing color patterns. *Developmental genetics,* **15** : 79-91.

RANK, N.E. (1992) – A hierarchical analysis of genetic differentiation in a montane leaf beetle *Chrysomela aeneicollis* (Coleoptera : Chrysomelidae). *Evolution,* **46** : 1097-1111.

SILFVERBERG, H. (1991) – Colour variation in Finnish Chrysomelidae (Coleoptera). 1. Cryptocephalinae. *Elytron suppl.,* **5**(1): 149-157.

SILFVERBERG, H. (1994) – Colour variation in Finnish Chrysomelidae (Coleoptera). 2 The genus *Gonioctena.* In : *Proceedings of the Third International Symposium of the Chrysomelidae Beijing, 1992.* FURTH, D.G. (Ed.). Backhuys Publishers, Leiden : 31-37.

SMITH, D.A.S., D.F. OWEN, I.J. GORDON & A.M. OWINY (1993) – Polymorphism and evolution in the butterfly *Danaus chrysippus* (L.) (Lepidoptera : Danainae). *Heredity,* **71** : 242-251.

SNEATH, P.H.A. & R.R. SOKAL (1973) – Numerical Taxonomy. W.H. Freeman and company, San Francisco : 573 pp.

SWOFFORD, D.L. & R.B. SELANDER (1989) – BIOSYS-1 : A computer program for the analysis of allelelic variation in population genetics and biochemical systematics. Illinois Natural History Survey, Illinois.

TER BRAAK, C.J.F. (1988) – CANOCO : a FORTRAN program for canonical community ordination by [partial] [detrended] [canonical] correspondence analysis, principal components analysis and redundancy analysis (version 2.1 and update notes version 3.1). Technical Report, Agriculture Mathematics Group, Wageningen.

THOMAS, S. (1964) – Some aspects of life history, genetics, distribution and taxonomy of *Aspidomorpha adhaerens* (Weber, 1801) (Cassidinae, Coleoptera). Ph. D. thesis, University of Amsterdam : 264 pp.

VASCONCELLOS-NETO, J. (1988) – Genetics of *Chelymorpha cribraria,* Cassidinae colour patterns and their ecological meanings. In : *Biology of Chrysomelidae.*P. JOLIVET, E. PETITPIERRE & T.H. HSIAO (Eds.). Kluwer Academic Publishers, Dordrecht : 217-232.

VERDYCK, P., T. BACKELJAU, K. BREUGELMANS & J. HULSELMANS (1992) – The use of isoelectric focusing to solve systematic problems in the genus *Phyllotreta* (Coleoptera, Chrysomelidae). *Belg. J. Bot.,* **125**(2): 310-314.

VERDYCK, P., L. DE BRUYN, J. HULSELMANS & J. SCHEIRS (1995) – Lectotype designation and discussion on the type material of three closely related Chrysomelid species : *Phyllotreta dilatata*

Thomson, 1866, *P. flexuosa* (Illiger, 1794) and *P. tetrastigma* (Comolli, 1837) (Col., Chrysomelidae). *Bull. Soc. ent. France,* **100**(3): 298.

VERDYCK, P., H. DE WOLF, T. BACKELJAU & J. HULSELMANS (1996) – A genetic study of two colour forms of *Phyllotreta cruciferae* (Chrysomelidae: Alticinae). In: *Chrysomelidae Biology, vol. 1: The classification, phylogeny and genetics.* P.H.A. JOLIVET & M.L. COX (Eds.). SPB Academic Publishing, Amsterdam, The Netherlands: 389-397.

VERDYCK, P., L. DE BRUYN & J. HULSELMANS (in press.) – A morphological study of *Phyllotreta tetrastigma* (Coleoptera: Chrysomelidae: Alticinae) including two colour forms. *J. Nat. Hist.*

WRIGHT, S. (1965) – The interpretation of population structure by F-statistics with special regard to systems of mating. *Evolution,* **19**: 395-420.

WRIGHT, S. (1978) – Evolution and genetics of populations, vol. **4** Variability within and among natural populations. University of Chicago Press, Chicago: 580 pp.

DIETS OF ABUNDANT FISHES
FROM BEACH SEINE CATCHES IN SEAGRASS BEDS
OF A TROPICAL BAY (GAZI BAY, KENYA)

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Abstract. The composition of the diet of 14 fish species that were common in beach seine catches over the seagrass beds of Gazi Bay (Kenya) was investigated. Three trophic guilds could be distinguished based on dietary diversity and on the numerical and gravimetrical composition of the diet. *Herklotsichthys quadrimaculatus*, *Stolephorus indicus* and *Atherinomorus duodecimalis* were planktivores. Their stomach fulness index was low and the diet was not diverse. The main food items were harpacticoid and calanoid copepods and brachyuran zoea and megalopae. *Apogon thermalis*, *Fowleria aurita*, *Paramonacanthus barnardi*, *Mulloides flavolineatus*, *Lutjanus fulviflamma*, *L. argentimaculatus* and *Gerres acinaces* were benthivores, mainly feeding on small epi- and hyper-benthic prey. Their diet was very diverse and it was dominated by Amphipoda (Gammaridea), Tanaidacea and Mysidacea. Their fulness indices were low, but a little bit higher than those observed for the planktivores. A third group were the « piscivores » : *Bothus myriaster*, *Fistularia commersonii*, *Sphyraena barracuda* and *Plotosus lineatus*. The dominant items in the food spectrum of these species·were postlarval fishes and large nektonic invertebrates (gammaridean amphipods, mysids, shrimp and crabs). Their diet was not diverse and the fulness index was much higher than that of the other species examined. All other species caught were further classified according to the following feeding guilds : herbivores, planktivores, benthivores (epi- and hyperbenthivores) and piscivores. The ichthyofauna of Gazi Bay was clearly dominated by benthivores.

Key words : feeding ecology, trophic organization, fish, seagrass beds, Kenya

INTRODUCTION

This study presents data on the trophic organisation of the fish fauna of a shallow East-African bay (Gazi Bay, Kenya). The fish fauna of Gazi Bay has received consider-able attention in recent years (VAN DER VELDE *et al.*, 1994, DE TROCH *et al.*, 1996, KIMANI *et al.*, 1996, WAKWABI & MEES, unpublished data). For this study, fish were sam-pled in 9 stations with a beach seine over seagrass beds and unvegetated areas. A total of 3601 fishes belonging to 75 species and 40 families were caught (> 95% juveniles). Multivariate analysis of the catch data revealed that 3 communities could be distin-guished (DE TROCH *et al.*, 1996) : a first community occurred in the downstream part of

the river-fed western creek, were sandy bottoms with sparse seagrass vegetation occur. The fish community was characterised by low density and diversity and is not considered further. The two other communities were characterised by a high fish diversity. One community occurred in the upstream part of the western creek and was dominated by *Gerres acinaces* Bleeker, 1854 and *Atherinomorus duodecimalis* (Valenciennes, 1835). The other community was found in the shallow areas of the bay proper and in the mouth area of the eastern creek. There, the dominant species were *Apogon thermalis* Cuvier, 1829 and *G. acinaces* Bleeker, 1854.

Individuals in the dominant size-classes of the most abundant and characteristic species of these latter communities were selected for analysis of stomach contents. For the remaining fish species caught, information about their trophic guild was taken from the literature and from FISHBASE (1995).

The aim of this study was to investigate the diet of some common fishes whose diet is poorly documented to date and to get an idea of the trophic organisation of fishes in a typical East-African bay.
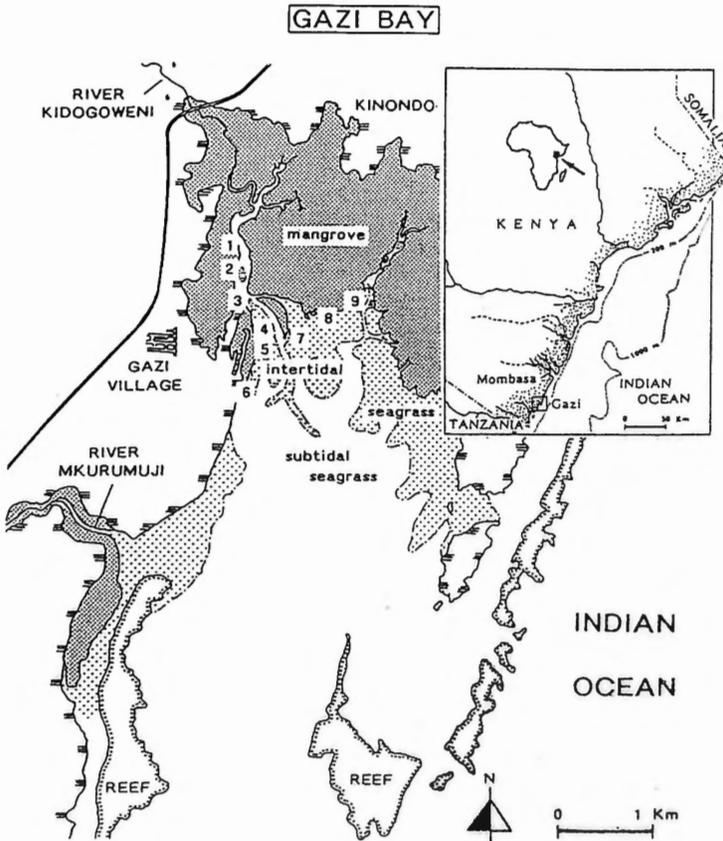
Fig. 1. – Map of Kenya with situation of Gazi Bay (COPPEJANS *et al.*, 1992). Detail of Gazi Bay (SLIM, 1995) with indication of the sampling stations.

MATERIAL AND METHODS

Study area

Gazi Bay (Kenya) is located some 50 km north of the Tanzanian border and 60 km south of Mombasa Island (4°22'S, 39°30'E). The bay is between 1.75 and 3.50 km wide and 3.25 km long and is bordered with mangroves. Two major creeks characterize the system (Fig. 1). The Kidogoweni river enters the bay through the so-called western creek (surface area ± 18 ha). The eastern creek (2.7 ha) has no freshwater input. In both major creeks and in the bay proper dense seagrass beds occur (percentage of cover between 30 and 100% in the creeks and 10 to 30% in the lagoon). The downstream part of the western creek is characterised by a sparse seagrass vegetation on a sandy bottom (SLIM, 1995).

Sampling

Samples were taken from two hours before to two hours after low-water springtide on the 17th (western creek) and 18th of August 1993 (eastern creek) with a beach seine net (1.20 m depth, 25 mm stretched mesh size). As the net was 80 m long, a single semi-circular haul was considered to sweep an area of about 509 m². All fish were immediately anaesthetized in a benzocaine solution (ethylamino-4-benzoate in seawater) to prevent regurgitation of the stomach content, and subsequently preserved in a 10% formaldehyde-seawater solution.

The location of the sampling stations is shown in Fig. 1. In each station, one sample was taken. Six stations were located in the western creek. The other three samples were taken in the intertidal and shallow subtidal seagrass beds in the eastern part of the bay (mouth of the eastern creek). The seagrass vegetation in each of these sampling stations is discussed by DE TROCH et al. (1996).

Diet analysis

In the laboratory all fishes were identified to species level using the keys provided by SMITH & HEEMSTRA (1986) and BIANCHI (1985). The number of individuals per species was counted and the standard length (SL) was measured to the nearest millimetre.

For all common species (>10 individuals) on which no dietary data was available, the length-frequency distribution was used to select the dominant length class. A list of species examined in this study, together with the length class and the sampling station is given in Table I.

A total of 456 fishes were selected for diet analysis. The fishes were dissected and the entire stomach was removed. For *Atherinomorus duodecimalis* (Valenciennes, 1835), *Mulloides flavolineatus* (Lacepède, 1801), *Fistularia commersonii* Rüppell 1838, *Gerres acinaces* Bleeker, 1854, *Lutjanus fulviflamma* (Forsskål, 1775) and *Lutjanus argentimaculatus* (Forsskål, 1775) the content of the stomach and the digestive tracts was considered as the stomach content *sensu lato*. All items present in the stomachs were identified to a high taxonomic level (Table II) and counted. The average number of prey (and prey biomass) per individual is indicated in the results as an indication for the difference in prey

TABLE II

List of the assigned biomass values, the length-ashfree dry weight (AFDW) and other morphometric regressions used to calculate the biomass of the different prey items. All lengths (L), total lengths (TL) and carapax width (CW) are in mm; all dry weights (DW), ashfree dry weights (AFDW) and assigned values are in mg

Nematoda	assigned value: 0.003
Foraminifera	assigned value: 0.001
Annelida	
Oligochaeta	$\ln AFDW = -6.030 + 1.813 \ln L$
Polychaeta	$\ln AFDW = -7.139 + 2.489 \ln L$
Mollusca	
Bivalvia	$\ln AFDW = -4.052 + 2.817 \ln L$
Crustacea	
Copepoda	
Calanoida	assigned value (adult): 0.016
Harpacticoida	assigned value (copepodite): 0.002
	assigned value (adult): 0.004
Ostracoda	assigned value: 0.014
Cladocera	*Daphnia* species: 0.01
Peracarida	
Cumacea	$\ln AFDW = -6.078 + 2.525 \ln TL$
Mysidacea	
Mesopodopsis spec.	$\ln AFDW = -6.107 + 2.867 \ln SL$
Other Mysidacea	$\ln AFDW = -6.120 + 2.994 \ln SL$
Isopoda (idem *Tardigrada*)	$\ln AFDW = -5.857 + 2.863 \ln TL$
Amphipoda	
Gammaridae	$\ln DW = -6.301 + 2.849 \ln SL$
Corophidae	$\ln DW = -6.435 + 2.681 \ln SL$
Other Amphipoda	$\ln AFDW = -5.857 + 2.863 \ln TL$
Tanaidacea	$\ln DW = -4.241 + 1.644 \ln SL$
Eucarida - Decapoda	
Caridea	
Crangon crangon	$\ln AFDW = -7.684 + 3.321 \ln TL$
	$TL = -0.6 + 8.7 AP$
	$TL = -0.4 + 3.82 CL$
	$TL = -0.4 + 6.1 TE$
Brachyura	
zoea	assigned value: 0.050
megalopa	assigned value: 0.189
adult	$\ln AFDW = -3.967 + 3.164 \ln CW$
Pisces	$\ln AFDW = -7.851 + 3.460 \ln SL$
fish eggs	assigned value: 0.025

A standardized way to measure or evaluate the weight (DW, dry weight) of the ingested food, is to express the amount of food as a percentage of the total fish weight, according to the formula for the fulness index (FI) defined by HUREAU (1969) (BERG, 1979):

$$FI = \frac{DW\ of\ stomach\ content}{total\ body\ DW} \times 100$$

To estimate the dry weight of the stomach content, this content was dried during 5 days at 60°C and weighted to 0.1 mg using a Sauter-balance.

The fulness index was not calculated for *Plotosus lineatus* (Thunberg, 1787) and *Sphyraena barracuda* (Walbaum, 1792) as the dry weight of these large species was not estimated. Empty stomachs were not included in the calculations.

To assess niche breadth the Shannon-Wiener diversity index (HILL, 1973) was calculated as:

$$H' = \sum_{i=1}^{n} p_i (\log p_i)$$

$$with\ p_i = \frac{N_i}{N_t} = relative\ abundance\ of\ prey\ item_i$$

RESULTS

Diet composition of dominant species

The stomach contents of the examined species are discussed in terms of numerical (%N) and gravimetrical (%G) percentages (Figs 2-3).

Herklotsichthys quadrimaculatus (Rüppell, 1837) (Blueline herring)

An average of 93 prey items was present in the ingested contents per individual. This average corresponds to a biomass of 3.3 mg AFDW per individual.

Numerically, harpacticoids were the dominant prey (69.7% of the total number of ingested prey). Other important prey items were brachyuran zoea larvae and Mollusca (mainly gastropods), which accounted for 9.3%N and 8.4%N, respectively. Ostracods (4.2%) and calanoid copepods (3.4%) were less important in the total food spectrum. Other prey items were brachyuran megalopa larvae (1.7%), isopods (1.4%N), tanaids (0.9%N) and gammaridean amphipods (0.3%N). Gravimetrically, the diet was dominated by megalopa larvae (54.2%G) and molluscs (17.3%G). The numerically dominant harpacticoids represented only 7.8% of the total amount of ingested biomass.
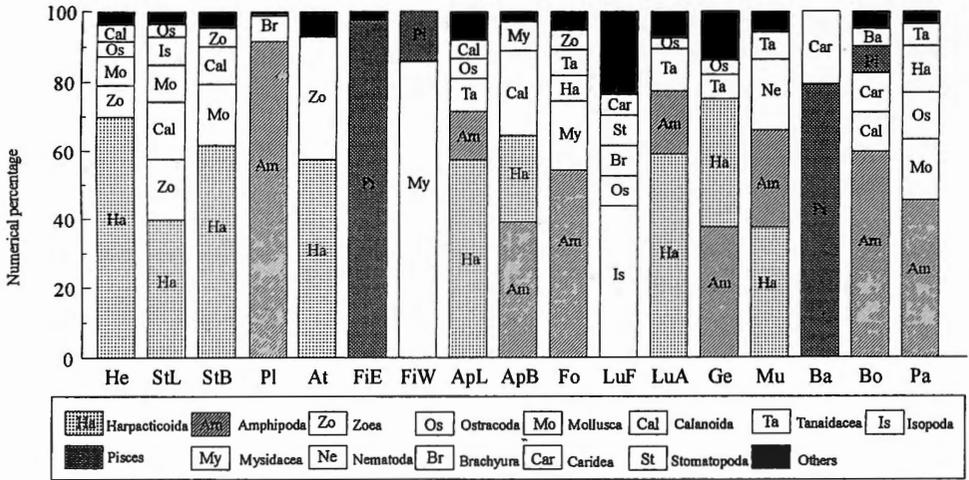
Fig. 2. – Numerical diet composition of the investigated fish species
(abbreviations see Tabel I)
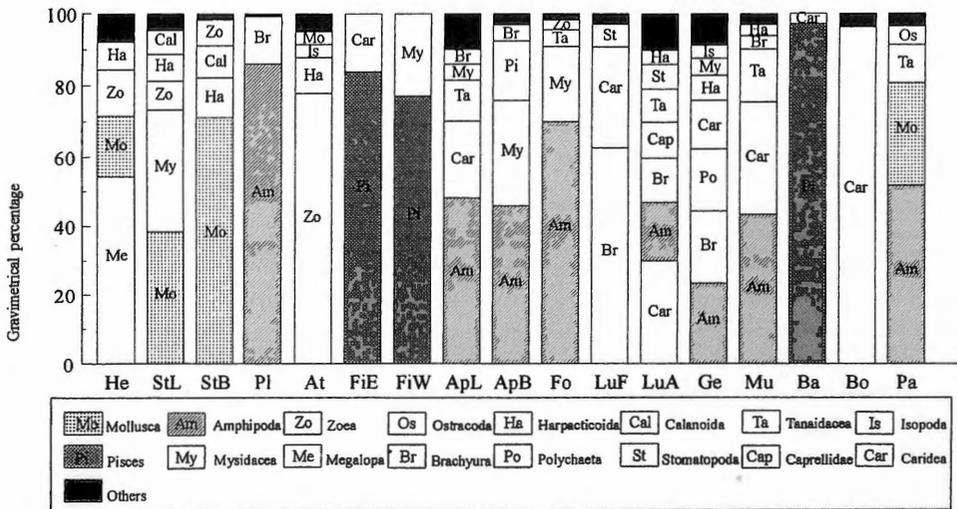
Fig. 3. – Gravimetrical diet composition of the investigated fish species
(abbreviations see Tabel I)

Stolephorus indicus (van Hasselt 1823) (Indian anchovy)

Since the length-frequency distribution of this species was clearly bimodal, individuals of two length classes were considered : 40-45 mm : StS (*Stolephorus indicus* small) and 70-75 mm : StL (*Stolephorus indicus* large).

An average number of 11.2 prey items was found in the individuals of the 40-45 mm length class. This corresponds to an average biomass of 0.6 mg AFDW. Individuals of the second length class contained an average of 38.4 prey and 0.46 mg AFDW per individual.

In both length classes harpacticoids were numerically dominant (39.8%N for StS, 61.7%N for StL). Other important prey were zoea larvae (17.7%N for StS, 5.2%N for StL), calanoids (16.8%N for StS, 10.7%N for StL) and molluscs (mainly gastropods) (10.6%N for StS, 17.7%N for StL). The diet resembles that of *Herklotsichthys quadrimaculatus* in terms of prey species, but not in the relative importance of each item *e.g.* calanoids were more important in the foodspectrum of *S. indicus*.

In terms of biomass, the Indian anchovy mainly utilised molluscs (gastropods) (38.4%G for StS, 71.0%G for StL). The main difference between both length classes was the contribution of mysids to the diet: these were absent from the stomachs of the larger (70-75 mm) individuals, while they formed an important prey (35%G) for the smaller (40-45 mm) individuals.

Plotosus lineatus (Thunberg 1787) (Striped eel-catfish)

An average of 37 prey items was found in the stomachs analysed. This corresponds to an average biomass uptake of 111 mg AFDW per fish. Numerically, the diet was dominated by amphipods (90.7%N) and Brachyura (8.4%N). The remaining prey (0.9%N) were Polychaeta and Caridea. The gravimetrical composition was also dominated by amphipods (85.6%G) and Brachyura (14.1%G). The other prey items counted for only 0.4% of the biomass.

Atherinomorus duodecimalis (Valenciennes 1835) (Tropical silverside)

An average of 170 prey items per individual was found, corresponding to an average biomass of 3.9 mg AFDW per fish. The food spectrum was dominated by harpacticoids (57.5%N) and zoea larvae (35.4%N). Calanoids (2.7%), isopods (1.4%), molluscs (1.3%) and prey species that were only occasionally found (foraminifers, ostracods, megalopa larvae, amphipods, brachyurans, tanaids, oligochaetes and shrimp) together accounted for 7.1%N of the diet.

Zoea larvae (77.6%G) dominated in gravimetrical terms. The tropical silverside contained an average of 3 mg AFDW of zoea larvae per individual. The numerically dominant harpacticoids represented 10.1% of the total gravimetrical composition. The other prey items were quite negligible in the total ingested biomass.

Fistularia commersonii (Rüppell 1838) (Smooth flutemouth)

The diets of individuals of the smooth flutemouth from the eastern creek (FiE) and the western creek (FiW) were compared.

In the analysis of the specimens from the eastern creek (FiE), an average of 4 prey items was found (14.8 mg AFDW). The dominant prey item was Pisces (97.6%N). Caridea and Amphipoda both accounted for 1.3%N. The importance of fish is also shown in the

gravimetrical composition, where they constituted 83.7% of the ingested biomass. Caridea were more important gravimetrically (16.3%G) than numerically.

An average of 28 prey items per fish was found (18.1 mg AFDW) in the individuals from the western creek (FiW). Here mysids (86%N), 23.9 mysids per fish, dominated the diet. Pisces represented 14%N. The gravimetrical composition was similar to that of the individuals from the western creek. The major part was formed by fish (76.8%G). This corresponds to an average of 4.2 mg AFDW per individual. The remaining 23.2%G was mysids.

Apogon thermalis (Cuvier 1829) (Masked cardinal)

The individuals of the masked cardinal were taken from the eastern (30-33 mm standard length: ApE) and the western (35-38 mm standard length: ApW) creek.

In the individuals of the eastern creek, an average of 10.5 prey per individual was found (average biomass: 0.7 mg AFDW per fish). The masked cardinal fed primarily on harpacticoids (57.6%N). The other half of the diet consisted of gammaridean amphipods (13.9%N), tanaids (9.5%N), calanoids (5.7%N) and ostracods (5.1%). The 'other' prey (8.2%) were mysids, caridean shrimp, brachyuran crabs, isopods, Caprellidea, tardigrads and molluscs. The gravimetrical composition was dominated by Amphipoda (48.1%G) and Caridea (21.9%G).

The individuals of the western creek contained an average of 9 prey items per individual (0.9 mg AFDW per fish). Compared to the individuals of the eastern creek, the same prey items were consumed but amphipods (39.3%N) were the most important prey. Half of the diet was numerically composed of harpacticoids (25.2%) and mysids (24.4%).

Gravimetrically, the diet was also dominated by amphipods (45.7%G) but mysids (29.9%G) replaced the Caridae from the diet of individuals from the eastern creek . Pisces accounted for 16.6% of the ingested biomass, but were numerically low.

Fowleria aurita (Valenciennes 1831) (Crosseyed cardinal)

An average of 3.7 prey per fish were counted (0.6 mg AFDW per fish). Amphipods were numerically dominant (54.5%N). Mysids accounted for 20% of the total number of ingested prey. Tanaids and harpacticoids both represented 7.3%N, while the numerical percentage of the zoea larvae was 5.5%. The diet was supplemented with calanoids, Caridea and Polychaeta.

The gravimetrical composition emphasizes the importance of amphipods (69.7%G) in the diet. Mysidacea are the second most important source of energy (20.9%G) and tanaids represented 4.7%G.

Lutjanus fulviflamma (Forsskål 1775) (Dory snapper)

Very few prey items (average of 3 per individual) were found per fish, corresponding to an average biomass uptake of 11.9 mg AFDW.

The diet of *L. fulviflamma* (Dory snapper) was numerically dominated by isopods (mainly Sphaeromatidae): 44.1%N. Other important prey were ostracods (8.8%N), brachyurans (8.8%N), stomatopods (8.8%N) and caridean shrimp (5.9%N). The 'other' prey were mainly polychaetes (2.9%N) and unidentified crustacean material (1.1%N).

The gravimetrical composition was principally brachyurans (62.3%G), shrimp (27.3%G) and stomatopods (6.4%G).

Lutjanus argentimaculatus (Forsskål 1775) (River snapper)

An average of 22 prey items per fish was found, corresponding to a biomass of 1.2 mg AFDW.

L. argentimaculatus mainly fed on harpacticoids (59.3%N). A smaller percentage was covered by amphipods (18.1%N), tanaids (12.0%N) and ostracods (3.0%N).

Gravimetrically, the important food sources were shrimp (29.8%G), amphipods (16.8%G), brachyurans (12.7%G), caprellids (10.0%G), tanaids (9.3%G), stomatopods (6.8%G) and harpacticoids (4.2%G).

Gerres acinaces (Bleeker 1854) (Smallscale pursemouth)

The diet of the smallscale pursemouth, with an average of 31 prey items per individual (2.9 mg AFDW), was composed of amphipods (38.0%N), harpacticoids (37.1%N), tanaids (7.4%N) and ostracods (4.5%N). The 'other' prey were isopods, polychaetes and shrimps.

Gravimetrically, the diet is more diverse with prey-items like amphipods (23.6%G), brachyurans (20.8%G), polychaetes (19.2%G), shrimps (13.6%G), tanaids (7.0%G), mysids (4.7%G), isopods (3.8%G), megalopae larvae (2.1%G) and harpacticoids (1.6%G).

The stomach content of *G. acinaces* was characterised by high amounts of detritus (mainly fine macrophytal material) and sediment particles. An average of 85% of the stomach content weight was attributed to sediment and detritus.

Mulloides flavolineatus (Lacepède 1801) (Yellowstripe goatfish)

An average of 20 prey items per fish was found (0.96 mg AFDW). Numerically the diet of the yellowstripe goatfish was dominated by harpacticoids (37.9%N), amphipods (28.3%N) and nematodes (20%N). Other prey were tanaids (7.6%N), ostracods (2.0%N), brachyurans (1.5%N), Caridea (1.0%N), isopods (1.0%N) and polychaetes (0.5%N).

In gravimetrical terms amphipods dominated as they accounted for almost half (43%G) of the ingested biomass. The numerically low Caridea, constituted 31.8%G of the gravimetrical composition. Nematodes were gravimetrically insignificant (1.2%G).

Sphyraena barracuda (Walbaum 1792) (Great barracuda)

An average of only 1 prey item per fish was found. Still, the average biomass uptake was 1327.3 mg AFDW per individual. Both numerically and gravimetrically (79.3% N

and 97% G), the dominant prey items were Pisces. The diet was supplemented with caridean shrimp (20%N, 3%G).

Bothus myriaster (Temminck & Shlegel 1846) (Disc flounder)

An average of 8 prey items was found per fish (68.5 mg AFDW). Amphipods (60%N) dominated the diet. Calanoids and brachyurans were of secondary importance (both 11%N). Other prey items were Caridea, Pisces, Harpacticoida and Cumacea. Gravimetrically, the diet was dominanted by Caridea (96%G).

Paramonacanthus barnardi (Fraser-Brunner 1941) (Wedgetail filefish)

An average number of 45 prey items and 2.1 mg AFDW was found. Numerically, amphipods dominated the diet (46.1%N). This percentage corresponds to an average of 20 amphipods per fish. The second important prey were gastropod molluscs (almost one fifth). Ostracods and harpacticoids were less important (both 13%N). Other prey were tanaids, isopods, caprellids, foraminifers, tardigrads, calanoids, shrimp, brachyuran zoea larvae and nematodes.

Amphipods (51.5%G) were also dominant in the gravimetrical composition. Molluscs represented one fourth of the gravimetrical composition.

Others

For the remaining species caught the trophic guild to which they belong is given in Table III. This classification is based on information available in the literature and in FishBase (1995).

TABLE III

*Species list with number of individuals in the communities of the western (N_{ind} west)
and eastern (N_{ind} west) creeks and both communities pooled (N_{total})
together with the trophic guild*

species	N_{total}	N_{ind} west	N_{ind} east	trophic guild	source
Gerres acinaces	1095	917	178	benthivore	SMITH & HEEMSTRA (1986), present study
Atherinomorus duodecimalis	622	622	-	planktivore	present study
Apogon thermalis	228	21	207	benthivore	present study
Herklotsichthys quadrimaculatus	227	225	2	planktivore	MILTON et al. (1994), present study
Stolephorus indicus	128	128	-	planktivore	WHITEHEAD (1985), present study
Fowleria aurita	100	85	15	benthivore	SANO et al. (1984), present study
Plotosus lineatus	88	88	-	piscivore	VAN WAEYENBERG (1994), present study

species	N_{total}	N_{ind} west	N_{ind} east	trophic guild	source
Lutjanus argentimaculatus	87	33	54	benthivore	KULBICKI *et al.* (1993), present study
Leptoscarus vaigiensis	60	2	58	herbivore	SOUSA & DIAS (1981)
Lethrinus lentjan	60	15	45	benthivore	CARPENTER & ALLEN (1989)
Scarus ghobban	55	6	49	herbivore	SANO *et al.* (1984), ANDERSON & HAFIZ (1987)
Lutjanus fulviflamma	53	21	32	benthivore	SANO *et al.* (1984), present study
Scarus spec.	53	42	11	herbivore	SMITH & HEEMSTRA (1986)
Siganus sutor	46	23	23	herbivore	WOODLAND (1990), ROBINS *et al.* (1991)
Fistularia commersonii	46	13	33	piscivore	present study
Leiognathus fasciatus	41	41	-	benthivore	BLABER (1980), FISCHER *et al.* (1990)
Paramonacanthus barnardi	35	7	28	benthivore	present study
Petroscirtes mitratus	28	15	13	herbivore	SANO *et al.* (1984)
Petroscirtes breviceps	28	1	27	herbivore	SANO *et al.* (1984)
Parupeneus barberinus	22	17	5	benthivore	SANO *et al.* (1984)
Sphyraena barracuda	20	18	2	piscivore	RANDALL (1967), present study
Stethojulis strigiventer	19	1	18	benthivore	SANO *et al.* (1984)
Amblygobius albimaculatus	17	3	14	herbivore	SANO *et al.* (1984)
Parascorpaena mossambica	14	9	5	unknown	-
Mulloides flavolineatus	13	2	11	benthivore	present study
Syngnathoides biaculeatus	11	-	11	planktivore	SMITH & HEEMSTRA (1986)
Cheilio inermis	10	-	10	piscivore	SANO *et al.* (1984)
Bothus myriaster	10	10	-	piscivore	present study
Cheilodipterus quinquelineatus	8	-	8	benthivore	SANO *et al.* (1984), PAXTON *et al.* (1989)
Ablennes hians	8	8	-	piscivore	FISCHER *et al.* (1990)
Sebastapistes strongia	7	7	-	unknown	-
Pterois miles	5	1	4	unknown	-
Oplopomus oplopomus	6	-	6	benthivore	SANO *et al.* (1984)
Synodus variegatus	6	5	1	benthivore	PAULIN *et al.* (1989)
Gazza minuta	4	4	-	piscivore	BLABER (1980)
Solenostomus cyanopterus	4	-	4	planktivore	MYERS (1991)
Pelates quadrilineatus	4	-	4	benthivore	SMITH & HEEMSTRA (1986)
Platax teira	3	2	1	unknown	-
Tylosurus crocodilus crocodilus	1	-	1	piscivore	RANDALL (1967), BLABER (1980)
Canthigaster bennetti	3	1	2	herbivore	MYERS (1991)

species	N_{total}	N_{ind} west	N_{ind} east	trophic guild	source
Aluterus scriptus	3	-	3	benthivore	RANDALL (1967), MYERS (1991)
Alectis indicus	3	3	-	piscivore	FISCHER & BIANCHI (1984), FISCHER *et al.* (1990)
Cheilinus chlorourus	2	2	-	benthivore	SANO *et al.* (1984)
Gerres rappi	2	-	2	benthivore	WOODLAND (1984)
Upeneus tragula	2	2	-	benthivore	SANO *et al.* (1984)
Ostracion cubicus	2	2	-	herbivore	MYERS (1991), CORNIC (1987)
Gerres filamentosus	2	2	-	benthivore	BLABER (1980)
Lethrinus harak	2	2	-	benthivore	CARPENTER & ALLEN (1989)
Asterropteryx semipunctatus	2	-	2	herbivore	SANO *et al.* (1984)
Liza macrolepis	2	2	-	herbivore	SKELTON (1993), THOMSON & LUTHER (1984)
Dendrochirus brachypterus	2	2	-	unknown	-
Arothron meleagris	2	2	-	herbivore	RANDALL (1985), GUZMAN & LOPEZ (1991)
Trachyrhamphus bicoarctatus	2	2	-	benthivore	SMITH & HEEMSTRA (1986)
Leiognathus elongatus	1	1	-	benthivore	JAMES (1984)
Lactoria fornasini	1	1	-	unknown	-
Caranx sexfasciatus	1	1	-	piscivore	HONEBRING (1990), SALINI *et al.* (1994)
Epinephelus spec.	1	-	1	unknown	-
Dactyloptena orientalis	1	1	-	unknown	-
Naso brevirostris	1	1	-	herbivore	RANDALL (1985)
Parupeneus macronema	1	-	1	benthivore	FISCHER *et al.* (1990)
Platax orbicularis	1	1	-	unknown	-
Aulostomus chinensis	1	-	1	piscivore	RANDALL (1985)
Aeoliscus punctulatus	1	-	1	planktivore	SMITH & HEEMSTRA (1986)
Platycephalus indicus	1	-	1	piscivore	FISCHER *et al.* (1990)
Chaetodon xanthocephalus	1	-	1	herbivore	CORNIC (1987)
Diagramma pictum	1	-	1	benthivore	JONES *et al.* (1992)
Tylerius spinosissimus	1	-	1	unknown	-
Oligolepis acutipennis	1	-	1	benthivore	SANO *et al.* (1984)
Arothron immaculatus	1	1	-	herbivore	RANDALL (1985)
Neopomacentrus cyanomos	1	1	-	herbivore	PARRISH (1989)

Fulness index (FI)

The mean fulness indices together with the standard errors are shown in Fig. 4.
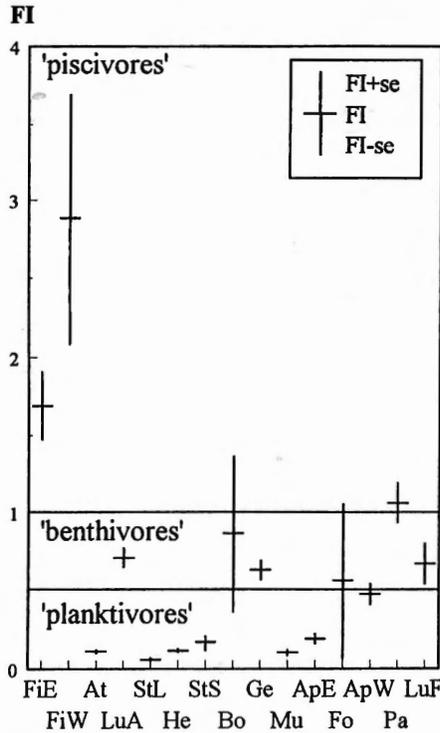
Fig. 4. – Fulness index (FI) together with the standard error (se)

For most species the fulness index was lower than 0.5: *Atherinomorus duodecimalis* (FI=0.109±0.008), *Stolephorus indicus* (70-75mm) (FI=0.060±0.011), *Herklotsichthys quadrimaculatus* (FI=0.115±0.009), *S. indicus* (40-45 mm) (FI=0.172±0.056), *Mulloides flavolineatus* (FI=0.102±0.016), *Apogon thermalis* (30-33mm) (FI=0.189±0.031), *A. thermalis* (35-38mm) (FI=0.473±0.066).

Five species had a fulness index between 0.5 and 1: *Lutjanus argentimaculatus* (FI=0.71±0.06), *Bothus myriaster* (FI=0.864±0.502), *Gerres acinaces* (FI=0.63±0.06), *Fowleria aurita* (FI=0.561±0.497) and *L. fulviflamma* (FI=0.67±0.13).

The fulness index of the other species had a value between 1 and 3: *Fistularia commersonii* in the eastern creek (FI=1.692±0.218) and in the western creek (FI=2.889±0.802) and *Paramonacanthus barnardi* (FI=1.066±0.129).

Diversity of the diet

The diet was most diverse for *Lutjanus fulviflamma* (H'=0.89), *Paramonancanthus barnardi* (H'=0.81), *Apogon thermalis* (35-38 mm) (H'=0.79), *Fowleria aurita* (H'=0.77), *Apogon thermalis* (33-36 mm) (H'=0.74), *Mulloides flavolineatus* (H'=0.72) and *Gerres acinaces* (H'=0.71) (Fig. 5). This group corresponds to the species that mainly fed on benthic (hyper- and epibenthic) prey. A second group was characterised by a lower dietary diversity ranging from 0.4 to 0.55 : *Atherinomorus duodecimalis* (H'=0.44), *Lutjanus fulviflamma* (H'=0.45), *Stolephorus indicus* (70-75mm) (H'=0.51) and *Herklotsichthys quadrimaculatus* (H'=0.53). This group mainly fed on harpacticoids (except *Lutjanus fulviflamma*) and were considered to be 'planktivores' in this study. The diet of two species had an intermediate diversity : *Stolephorus indicus* (40-45mm) (H'=0.64) and *Bothus myriaster* (H'=0.66). The 'piscivores' *Plotosus lineatus* (H'=0.15), *Fistularia commersonii* (eastern creek) (H'=0.06), *F. commersonii* (western creek) (H'=0.18) and *Sphyraena barracuda* (H'=0.22) had the least diverse diet.
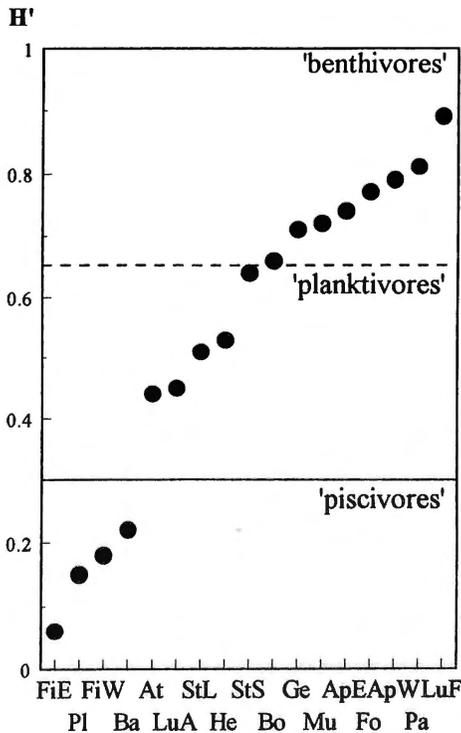
Fig. 5. – Diversity of the diet (Shannon-Wiener index H')

DISCUSSION

Based on the numerical and gravimetrical composition of the diet, the fulness index and the diversity of the diet four guilds (SIMBERLOFF & DAYAN, 1991) could be distinguished. *Herklotsichthys quadrimaculatus*, *Stolephorus indicus* and *Atherinomorus duodecimalis* were planktivores which mainly fed on harpacticoid and calanoid copepods and brachyuran zoeae and megalopae. They were characterised by a low fulness index and an average diversity of the diet of 0.53. These species mainly fed on planktonic prey. Harpacticoid copepods may also have been taken from the leaves of seagrasses, where they are very abundant (DE TROCH, unpublished data). The guild of the planktivores thus needs a broad interpretation. Species feeding on epiphytic organisms were also included in this guild. PARRISH (1989) separated the planktivores based on whether they feed on pelagic holoplankton or « demersal » meroplankton. VAN DER VELDE *et al.* (1994) used the guild planktivore/benthivore for species feeding on merozooplankton and benthic organisms.

The diet of *Apogon thermalis*, *Fowleria aurita*, *Paramonacanthus barnardi*, *Mulloides flavolineatus*, *Lutjanus argentimaculatus*, *L. fulviflamma* and *Gerres acinaces* was mainly composed of amphipods, tanaids and mysids. On average, their diet was very diverse (H'=0.74) and the fulness index was intermediate. They were considered to belong to the guild of the « benthivores ».

The data suggest that this guild can actually be divided in 3 subguilds, based on the sub-compartment of the benthos they preferentially utilise. Species feeding predominantly on mysids and amphipods (e.g. *Apogon thermalis*, *Fowleria aurita*) can be considered to be « hyperbenthivores » i.e. they feed in the water layers close to the substratum (the uppermost benthic compartment or hyperbenthal) where these taxa are known to occur abundantly (MEES & JONES, 1997; MEES, unpublished data). Species like *Paramonacanthus barnardi*, *Mulloides flavolineatus*, *Lutjanus argentimaculatus* and *L. fulviflamma* mainly consume tanaids, amphipods, isopods, molluscs, ostracodes, polychaetes... and can be considered to be « epibenthivores ». They feed on taxa that live in close association with the substratum or that are attached to the seagrasses. *Gerres acinaces* is an « endobenthivore », as shown by the high amounts of sediment in their stomach. They take their prey by filtering the sediment through the gills.

The food composition of *Plotosus lineatus*, *Fistularia commersonii*, *Sphyraena baracuda* and *Bothus myriaster* was dominated by fish and nektonic macrocrustaceans (caridean shrimp, large amphipods, crabs and mysids). Their diet had a very low diversity (average H'=0.15) and the fulness index was higher than that of the other species examined. PARRISH (1989) also used the guild of piscivores, while MORTON (1990) made a distinction between intermediate carnivores (feeding on macrobenthos and small fishes) and topcarnivores (exclusively feeding on fishes).

The trophic guild of « herbivores » (not encountered during the stomach analysis performed for this study) is broad and can also be divided into several subguilds. Species feeding on algae and seagrasses, as well as detritivores and corallivores were placed in this guild. Only juveniles of the corallivorous species were caught in the seagrass beds, where they are supposed to feed on non-corallivorous material. Some scientists have approached

this problem by lumping herbivores and coral feeders together (PARRISH, 1989). « Non-car-nivore » would be a better term instead of « herbivore ».

In the community of the western creek, half of the individuals (49%) were benthivores (Fig. 6A). This is correlated with the high densities of *Gerres acinaces* and *Lutjanus argentimaculatus*. Also planktivores were important in this community (40%). In terms of number of species (Fig. 6B), the community was also dominated by benthivores (37%). The high density of planktivores was attributable to a low number of species (7% of the total number of species in the community). This can be explained by the monospecific schooling behaviour of species like *Herklotsichthys quadrimaculatus*, *Stolephorus indicus* and *Atherinomorus duodecimalis*.

A

40.55%   2.55%
                                                    22.27%

4.22%
0.99%   1.34%
5.79%   5.35%

                    68.49%

48.45%

western creek   eastern creek

■ herbivores ▥ planktivores □ benthivores ▦ piscivores ▨ unknown

B

7.35%   6.58%
        26.47%   23.68%

36.76%   43.42%

        14.71%   13.16%

14.71%   13.16%

western creek   eastern creek

■ herbivores ▥ planktivores □ benthivores ■ piscivores ▨ unknown
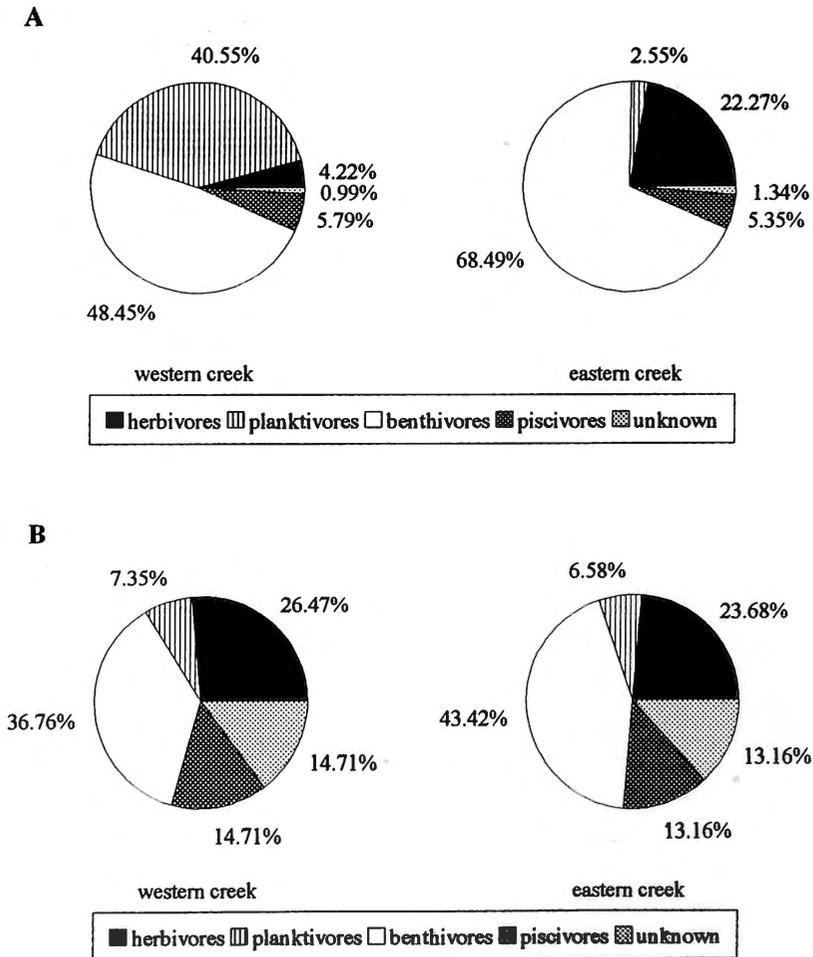
Fig. 6. – Trophic composition of the ichthyofauna occurring in the eastern and the western creek : (A) based on the densities, (B) based on the number of the species.

The fish community of the eastern creek was also dominated by benthivores (69% of the total density, Fig. 6A), corresponding to 43% of the total number of species (Fig. 6B). In this community, the densities and number of herbivorous species were remarkably higher (22% of the total density, 24% of the number of species) than in the community occurring in the western creek (less than 5% of the total density but 26% of the number of species).

It should be stated that the data obtained in this study were based on a single sampling campaign where mainly juvenile fishes were caught. The results and conclusions characterizing different guilds are thus based on dietary information for juvenile fishes. The same is true for the relevance of using niche breadth and fulness index. Additional data from a temporal study are currently being analyzed by E.O. Wakwabi.

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REFERENCES

ANDERSON & HAFIZ (1987) – *Common reef fishes of the Maldives*. Novelty Press, Republic of Maldives.

BERG, J. (1979) – Discussion of methods of investigating the food of fishes, with reference to a primary study of the prey of *Gobiusculus flavescens* (Gobiidae). *Mar. Biol.*, **50**: 263-273.

BIANCHI, G. (1985) – FAO species identification sheets for fishery purposes. *Field guide on the commercial marine and brackish-water species of Tanzania*. Food and agriculture organisation of the United Nations, Rome.

BLABER, S.J.M. (1980) – Fish of the Trinity Inlet Systems of North Queensland with notes on the ecology of fish faunas of tropical Indo-Pacific estuaries. *Austr. J. Mar. Freshw. Res.*, **31**: 137-146.

CARPENTER, K.E. & G.R. ALLEN (1989) – FAO species catalogue. Vol. 9. Emperor fishes and large-eye breams of the world (family Lethrinidae). An annotated and illustrated catalogue of Lutjanid species known to date. *FAO Fish. Synop.*, **125** (9) (118 pp).

COPPEJANS, E., T. BEECKMAN & M. DE WIT (1992) – The seagrasses and associated macroalgal vegetation of Gazi Bay (Kenya). *Hydrobiologia*, **247**: 59-75.

CORNIC, A. (1987) – *Poissons de l'Ile Maurice*. Editions de l'Océan Indien. Stanly Rose Hill, Ile Maurice (335 pp).

DE TROCH, M., J. MEES, I. PAPADOPOULOS & E. WAKWABI (1996) – Fish communities of Gazi Bay (Kenya): seagrass beds vs. unvegetated areas. *Neth. J. Zool.*, **46** (3-4): 236-252.

FISHBASE (1995). *FishBase: a biological database on fish*. Version 1.2. CD-ROM, ICLARM, Manila.

FISCHER, W. & G. BIANCHI (eds.) (1984) – FAO species identification sheets for fishery purposes. Western Indian Ocean (Fishing Area 51). Vol. 2. (pag. var.). FAO, Rome.

FISCHER, W., I. SOUSA, C. SILVA, A. DE FREITAS, J.M. POUTIERS, W. SCHNEIDER, T.C. BORGES, J.P. FERAL & A. MASSINGA (1990) – Fichas FAO de identificaçao de espécies para actividades de pesca. Guia de campo das espécies comerciasis marinhas e de águas salobras de Mocambique. Publicaçao preparada em collaboraçao com o Instituto de Investigaçao Pesquiera de Moçambique, com financiamento do Projecto PNUD/FAO MOZ/86/030 e de NORAD. Roma, FAO. (424 pp).

- GUZMAN, H.M. & J.M. LOPEZ (1991) – Diet of the corallivorous pufferfish *Arothron meleagris* (Pisces : Tetradontidae) at Gorgona Island, Colombia. *Revista de Biologia tropical,* **39** (2) : 203-206.

HILL, M.O. (1973) – Diversity and evenness : a unifying notation and its consequences. *Ecology,* **54** : 427-432.

HONEBRING, R. (1990) – Fishing in Hawaii : a student manual. Education Program, Hawaii. Division of Aquatic Resources. (79 pp).

HUREAU, J.C. (1969) – Biologie comparée de quelques poissons antarctique (Nothotheniidae). *Bulletin de l'Institute océanographique de Monaco,* **68** : 1-44.

JAMES, P.S.B.R. (1984) – Leiognathidae. In *FAO species identification sheets for fishery purposes. Western Indian Ocean (Fishing Area 51). Vol. 2. (pag. var.). FAO, Rome* (Fischer, W. & Bianchi, G., eds.). Prepared and printed with the support of the Danish International Development Agency (DANIDA). FAO, Rome, Vol. 1-6, pp. var.

JONES, G.P., D.J. FERRELL & P.F. SALE (1992) – Fish feeding and dynamics of soft–sediment mollusc populations in a coral reef lagoon. *Mar. Ecol. Progr. Ser.,* **80** : 175-190.

KIMANI, E.N., G.K. MWATHA, E.O. WAKWABI, J.M. NTIBA & B. OKOTH (1996). Fishes of a shallow tropical mangrove estuary, Gazi, Kenya. *Mar. Freshw. Res.,* **47** : 857-868.

KULBICKI, M., M. MOU THAM, P. THOLLOT & L. WANTIEZ (1993) – Length-weight relationships of fish from the lagoon of New Caledonia. NAGA, The ICLARM Q., **16** (2-3) : 26-29.

MEES, J., 1994. Het hyperbenthos van ondiepe kustgebieden en estuaria : gemeenschapsstruktuur en biologie van de dominante soorten. PhD. Universiteit Gent. 212 pp.

MEES, J. & M.B. JONES (1997) – The hyperbenthos. *Ocenaography and Marine Biology : an Annual Review,* **35** : 221-255.

MILTON, D.A., S.J.M. BLABER & N.J.F. ROWLINSON (1994) – Diet, prey selection, and their energetic relationship to reproduction in the tropical herring *Herklotsichthys quadrimaculatus* in Kiribati, Central Pacific. *Mar. Ecol. Prog. Ser.,* **103** : 239-250.

MORTON, R.M. (1990) – Community structure, density and standing crop of fishes in a subtropical Australian mangrove area. *Mar. Biol.,* **105** : 385-394.

MYERS, R.F. (1991) – *Micronesian reef fishes.* Second Ed. Coral Graphics, Barrigada, Guam. 298 pp.

PARRISH, J.D. (1989) – Fish communities of interacting shallow-water habitats in tropicaloceanic regions. *Mar. Ecol. Prog. Ser.,* **58**, 143-160.

PAULIN, C., A. STEWART, C. ROBERTS & P. MCMILLAN (1989) – *New Zealand fish : a complete guide.* National Museum of New Zealand Miscellaneous Series, **5** (279 pp).

PAXTON, J.R., D.F. HOESE, G.R. ALLEN & J.E. HANLEY (1989) – *Pisces. Petromyzontidae to Carangidae.* Zoological Catalogue of Australia, Vol. 7. Australian Government Publishing Service, Canberra (665 pp).

RANDALL, J.E. (1967) – Food habits of reef fishes of the West Indies. *Stud. Trop. Ocean. Miami,* **5** : 665-847.

RANDALL, J.E. (1985) – *Guide to Hawaiian reef fishes.* Harrowood Books, Newtown Spuare, PA 19073 USA.

ROBINS, C.R., R.M. BAILEY, C.E. BOND, J.R. BROOKER, E.A. LACHNER, R.N LEA & W.B. SCOTT (1991) – World fishes important to North Americans. Exclusive of species from the continental waters of the United States and Canada. *American Fish. Soc. Spec. Publ.,* **21** (243 pp).

SALINI, J.P., S.J. BLABER & D.T. BREWER (1994) – Diets of trawled predatory fish of the Gulf of Carpentaria, Australia, with particular reference to predation on prawns. *Austr. J. Mar. Freshw. Res.,* **45** : 397-411.

SANO, M., M. SHIMIZU & Y. NOSE (1984) – *Food habitats of teleostean reef fishes in Okinawa Island, Southern Japan.* University of Tokyo Press, Tokyo, Japan, (128 pp).

SIMBERLOFF, D.& T. DAYAN (1991)–The guild concept and the structure of ecological communities. *Annual review of Ecology and Systematics,* **22** : 115-143.

SKELTON, P.H. (1993) – *A complete guide to the Freshwater Fishes of Southern Africa.* Southern Book Publishers (Pty) Ltd. (388 pp).

SLIM, F.J. (1995) – *In : Interlinkages between Eastern-African coastal ecosystems.* Fourth semi-annual report on the E.C.-STD-3-project, contract TS3-TS 92-0114.

SMITH, M.M. & P.C. HEEMSTRA (eds.) (1986) – *Smith's sea fishes.* London : Springer-Verlag. (1047 pp).

SOUSA, M.I. & M. DIAS (1981) – *Catálago de peixes de Moçambique – Zona Sul.* Instituto de Desenvolvomento Pesqueiro, Maputo. (121 pp).

THOMSON, J.M. & G. LUTHER (1984) – Mugilidae. *In : FAO species identification sheets for fishery purposes. Western Indian Ocean (Fishing Area 51).* (FISCHER, W. & G. BIANCHI, eds.) Vol. 3. (pag. var.), FAO, Rome.

VAN DER VELDE, G., P.H. VAN AVESAATH, M.J. NTIBA, G.K. MWATHA, S. MARGUILLER & A.F. WOITCHIK (1994) – Fish fauna of mangrove creeks, seagrass meadows and sand flats in Gazi Bay : a study with nets and stable isotopes. In : *Interlinkages between Eastern-African Coastal Ecosystems,* second semi-annual report on the E.C. STD-3 project (M.A. HEMMINGA) : 28-37. Netherlands Institute of Ecology, Center for Estuarine and Coastal Ecology, Yerseke, The Netherlands.

WHITEHEAD, P.J.P. (1985) – *FAO species catalogue. Vol. 7. Clupeid fishes of the world. An annotated and illustrated catalogue of the herrings, sardines, pilchards, sparts, shads, anchovies and wolfherrings. Part 2* - Engraulidae. Fao Fish. Synop. **7 (125)** Pt. 2 : 579 pp.

WOODLAND, D.J. (1984) – Gerreidae. In *FAO species identification sheets for fishery purposes. Western Indian Ocean (Fishing Area 51). Vol. 2.(pp. var.). FAO, Rome* (FISCHER, W. & BIANCHI, G., eds.). Prepared and printed with the support of the Danish International Development Agency (DANIDA). FAO, Rome, Vol. 1-6, pp. var.

WOODLAND, D.J. (1990) – Revision of the fish family Siganidae with description of two new species and comments on distribution and biology. *Indo-Pacific Fishes,* **19** : 136 pp.

FACTORS
CHARACTERIZING THE DISTRIBUTION
OF THE SPOTLESS STARLING
(*STURNUS UNICOLOR* TEMMINCK)
IN CORSICA

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Abstract. The distribution of the Spotless Starling in Corsica was studied during the reproductive period of 1993 in order to determine the factors influencing the presence of breeding birds. We also tried to assess the number of reproducing pairs. The species is mainly found in intensively cultured pastures at low altitudes (below 860 m) with on average medium to high coverage of grazed area and where high and medium high maquis (wild scrub of Mediterranean lands) are scarce or absent. Although fruit cultures could be linked with the presence of reproducing birds, their influence appears to be of secondary importance. The number of reproducing birds in Corsica was estimated around 2500 pairs.

Key words : Spotless Starling, distribution, reproduction, agriculture.

INTRODUCTION

The Spotless Starling, *Sturnus unicolor* Temminck, 1820, is a west Mediterranean species present in Algeria, Tunisia, Morocco, the Iberian Peninsula and the larger west Mediterranean islands : Sicily, Sardinia and Corsica (CRAMP, 1994; YEATMAN-BERTHELOT, 1994). The Spotless Starling and the European Starling, *Sturnus vulgaris* Linnaeus, 1758, are sympatric in Catalonia (PERIS *et al.*, 1987, FERRER *et al.*, 1991). The sympatric area is still expanding through a constant progression of the Spotless Starling to the north. The species has been observed in France (Aude and Pyrénées orientales) since 1983 (CAMBRONY & MOTIS, 1994; KAYSER & ROUSSEAU, 1994; YEATMAN-BERTHELOT, 1994).

In Corsica the Spotless Starling is described as « common, but sparsely distributed » (YEATMAN, 1976; THIBAULT, 1983; CRAMP, 1994).

Our aim was to localise the actual breeding areas of the Spotless Starling throughout the island, look at densities and try to make an estimation of the Corsican population. We analysed the relationship of the birds with the agricultural land use.

METHODS

The study took place in 1993 from late March until mid-July. During the breeding season male Spotless Starlings sing very loudly. We mainly used this far-reaching song to determine the presence of starlings. We surveyed the different regions by car making regular stops of maximum 30 minutes duration. If no starlings were observed (binoculars 10X40) or heard during this period, we considered the species as absent. We revisited the regions where the Spotless Starling was noted absent, if previous reports mentioned the observation of breeding birds, if the region was neighbouring a colonised region or if the landscape offered apparently suitable habitats (absence of high dense vegetation). These searches took place between sunrise and 11.00 and from 17.00 until sunset, periods of enhanced activity (RENARD unpubl. data). Large forests (Vizzavona-Quensa-Ospedale) were excluded from our surveys because they did not offer any areas suitable for reproduction or feeding of the starling. Castagniccia was not surveyed because an intensive inventory of the region did not mention the presence of the Spotless Starling (PATRIMONIO, 1991). Castagniccia is mainly covered with chestnut forest (*Castanea sativa*), and offers very few open places for starlings to forage.

At each stop we described the possible presence of the Spotless Starling and the different landscape characteristics (see Table 1 and below for detailed description). Every observation was quantified in order to analyse the data statistically. Males were counted and categorised in four groups: no males present (= 0), isolated couple with one male (= 1), small colony composed of 10 males or less (= 2) and large colony with more than 10 males (= 3). The vicinity of other Spotless Starlings was described using the same categories according to the number of males observed during previous or following observation stops. We always used the highest observed neighbouring density in the analysis. For the presence of man-made constructions we used four groups. The division of the groups was based on the number of construction items. When no building was present, the landscape was categorised in category 0. One construction item consisting of a house with stables, stables alone, chapels, etc. was labelled category 1. Several houses with stables etc. not yet a village were categorised as 2. The last category (3) contained villages. (We defined a village as a central place surrounded by different houses.) Cities were categorised under villages, as the size difference was not always obvious and some cities actually consisted of several congregated villages. The observations were noted separately for ruins, old houses and recent constructions. We estimated the relative presence of the different types for villages and smaller construction groups. To describe the agricultural activities, we first divided the observations in two main groups: livestock and cultures. To determine the possible influence of farm animals we used the number of observed grazers during our search for starlings. In the analysis we made four categories according to the observed number and the species (2 groups). The observations ranged from absent (category 0), less than seven animals (category 1), small herd (counting possible, category 2) to large herd (counting not possible, category 3). For the species category goats and sheep were considered together as we mostly observed mixed herds, the other group contained cattle. Free roaming horses and pigs were not taken into account because of their small numbers. The vegetable, fruit and other cultures were noted if present (vineyard, orchard,

TABLE 1

Analysed environmental parameters noted during each observation stop

Groups	Observed parameter	Quantification
Birds	Estimation	0: absent 1: isolated couple (1 male) 2: small colony (2-10 males) 3: large colony (>10 males)
	Vicinity of other colonies	Number of observed males during previous or following observation stop. Categories as for estimation of bird numbers 0-3.
Constructions	Old Recent Ruin	0: no constructions 1: 1 item 2: small number (not a village) 3: village
Agricultural activity	Animals – sheep and goats – cattle	0: absent 1: less than 7 animals 2: small herd (counting possible) 3: large herd (counting not possible)
	Vineyard Orchard Olive grove Citrus Almond Kiwi Vegetables Maize Cereal Chestnut Hayfield Cork-tree	0: absent 1: present
Vegetation	Deciduous tree Conifer	0: absent 1: groups 2: groves 3: woody
	Grazing Intensively cultivated pasture Semi-intensive pasture with scrubs Wild pasture Low maquis (<50cm) Medium maquis (50cm-120cm) High maquis (>120cm)	0: <15% 1: 15%-50% 2: 50%-85% 3: 85%-100%
Geography	Altitude	Per 40 m

olive grove, citrus, almond, kiwi, vegetables, maize, cereal, chestnut, hayfield, cork-tree). The maquis vegetation was divided in three groups according to height: from low or medium to high maquis, not higher than approximately 50 cm, and 120 cm to higher than 120 cm. The presence of tree types (conifer and deciduous) ranged from complete absence (0) to groups (1), groves (2) and more or less woody (3). Grazing was quoted from 0 to 3. Zero corresponds with less than 15% grazed area, 1 with a grazed area between 15% and 50%, 2 with a grazed area between 50% and 85%, once higher than 85% we considered the area as completely grazed (=3). Pastures were categorised according to the degree of maintenance (three groups) into intensively cultivated, semi-intensively cultivated with some scrubs, and completely wild. The presence of the pastures in the landscape was described by four categories (0:<15%, 1:15%-50%, 2:50%-85%, 3: 85%-100%). We noted altitude per 40 m (topographic maps of the Institut Géographique National Paris).

For the estimation of population density we only considered reproducing birds. Non reproducing birds were not taken into account because they were difficult to assess for following reasons. If a bird is sexually active but does not succeed in attracting a mate it usually flies around prospecting possible mates or cavities. Such birds could be taken into account several times thus giving an overestimation of the number of Spotless Starlings. Other not sexually active birds could simply be overlooked as their behaviour is less conspicuous.

The impact of the individual factors on starling presence/absence was analysed with a t-test with logarithmic transformations for the continuous data (Basic Statistics/Tables module of STATISTICA 5.0 Statsoft 1994) or a P^2 -test for unordered 2xC tables for the categorical data (StatXact 3, METHA & PATEL, 1995). Sequential Bonferroni correction (RICE, 1989) was applied to prevent type I error due to multiple testing.

Because many factors can be interdependent, we applied logistic regression models (GLIM 4, FRANCIS et al,. 1994) to reveal the factors influencing the presence of breeding birds. A backward stepwise variable selection was utilised to obtain the minimal adequate model (CRAWLEY, 1993). We tested both the logit link-function and the complementary log-log link-function (FRANCIS et al., 1994). The categorical data were entered as factor variables in the model.

RESULTS

Using our observation of 1993 we compiled a distribution map of the Spotless Starling in Corsica (Fig. 1), projected on a map indicating roughly the main agricultural areas (ANON., 1989). We used the four categories described in the methods to indicate the colony size on the map. The nests could be found in natural as well as man made cavities (roofs, walls, etc.). Some isolated pairs in the surroundings of larger colonies are not mentioned on the map due to a lack of space. For the same reason dense vegetation patches (maquis) not colonised by the Spotless Starling but situated within densely populated areas were also omitted. Except for the Niolo, Corte and the basin of Ponte Leccia, the distribution of the Spotless Starling follows the coastal areas and incurving valleys. The distribution in coastal areas also coincides with rather flat areas
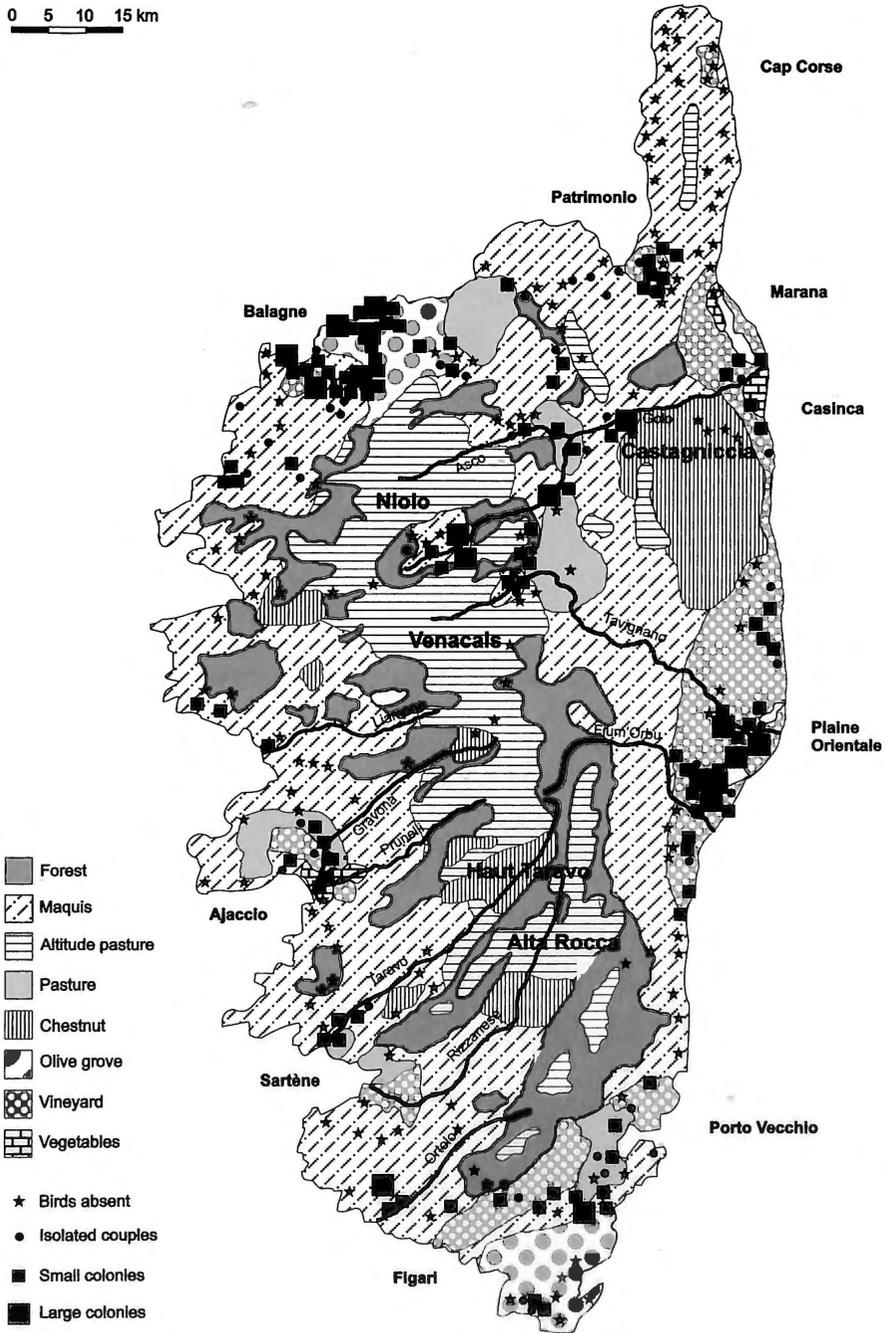
Fig. 1. – Distribution map of the Spotless Starling in Corsica during the breeding season of 1993.

(slopes less than 12%). The interior does not offer many plains, except for the Niolo. Here the Spotless Starling follows the less steep areas used as pastures. The highest Spotless Starling densities were found at the east coast and the Balagne; the Niolo can also be considered as a densely populated area as the birds were concentrated on a relatively small area (Calacuccia-Sidosi-Casamacioli). We estimated the total number of reproductive birds around 2500 pairs.

We analysed the factors determining the presence/absence of the Spotless Starling in Corsica during the reproductive period using all observation data of 1993 (Table 2). Some of the agricultural crops are only represented at a few localities (kiwi n=5, maize n=2, almond n=4) and could not be included in the analysis.

TABLE 2

*Impact of individual environmental parameters explaining Spotless Starling presence/absence. Data are summarised as mean ± SD. *: significant ($\alpha = 0.05$) after sequential Bonferoni) correction.*

Variables	birds absent	birds present	x^2	df	p
Vicinity	0.78 ± 0.92	1.94 ± 0.78	90.954	3	<0.001*
Old constructions	2.35 ± 0.94	1.70 ± 1.14	26.499	3	<0.001*
Recent construct.	1.01 ± 0.99	1.18 ± 0.99	5.168	3	0.160
Ruins	0.84 ± 0.47	0.74 ± 0.49	5.140	3	0.162
Vineyard	0.13 ± 0.34	0.34 ± 0.48	16.123	1	<0.001*
Olive groove	0.06 ± 0.23	0.21 ± 0.41	12.151	1	<0.001*
Citrus	0.02 ± 0.16	0.13 ± 0.33	9.224	1	0.002
Orchard	0.03 ± 0.18	0.11 ± 0.31	5.241	1	0.022
Hayfield	0.04 ± 0.20	0.12 ± 0.33	5.264	1	0.021
Grazing	1.12 ± 1.36	2.04 ± 0.85	75.598	3	<0.001*
Pasture cultured	0.38 ± 0.81	1.61 ± 1.29	65.293	3	<0.001*
Pasture semi-cult.	0.55 ± 0.89	1.38 ± 1.03	42.783	3	<0.001*
Pasture wild	0.62 ± 0.88	0.83 ± 0.92	4.958	3	0.175
Deciduous trees	0.67 ± 0.97	0.17 ± 0.47	28.274	3	<0.001*
Low maquis	1.12 ± 1.15	0.83 ± 0.96	22.501	3	<0.001*
Medium maquis	1.37 ± 1.25	0.24 ± 0.63	72.036	3	<0.001*
High maquis	1.07 ± 1.31	0.03 ± 0.26	73.741	3	<0.001*
Sheep	0.63 ± 1.04	0.21 ± 0.64	15.823	3	<0.001*
Cattle	0.39 ± 0.76	0.28 ± 0.62	5.777	3	0.123
			t	df	p
Altitude	239.59 ± 281.59	133.54 ± 180.65	3.667	270	<0.001*

The presence of reproducing birds is limited by altitude as we never observed fledgelings or singing males above 860 m (Albertacce, Niolo). Significantly more birds were present at lower altitudes. Observations of nesting starlings were significantly greater near other Spotless Starling colonies (vicinity), which indicates an aggregated distribution. Old villages are significantly less favourable for reproduction of the Spotless Starling whereas recent buildings and ruins can be considered as neutral.

Olive groves and vineyards positively influence the presence of reproducing Spotless Starlings. Other orchards seem less attractive for the Spotless Starling during reproduction, only citrus fruit showing a significant positive association with the birds. Starlings are observed significantly more often in the immediate surroundings of pastures. Regularly mowed grass or extensive grass management (free roaming cattle, sheep and goat) (pastures) are very likely to offer all requirements for optimal reproduction of the Spotless Starling. At intensively grazed areas, where short grassland dominates, Spotless Starlings are also significantly more likely to be present. Hayfields with alternately (twice a year) short and high grasses are not suitable for starlings. The absence of deciduous trees significantly influences the presence of Spotless Starlings. Medium or high scrub vegetation (maquis) also repels reproducing birds. Finally, the presence of starlings was significantly associated with herds of sheep and goats, but not with cattle.

When all parameters are combined in the logistic regression model to extract the minimal adequate model, five remain in the model (Table 3). The parameter vicinity was not included in the analysis because this parameter is more an indicator that the birds were observed in a larger suitable area than it is of direct influence on the presence of the observed colony. The model shows that Spotless Starlings mainly occur in the vicinity of intensively cultured pastures at low altitudes (below 860 m) with on average medium to high coverage of grazed area and where high and medium high maquis are scarce or absent (Fig. 2).

TABLE 3

Factors affecting the presence of breeding Starlings:
analysis of deviance table of the logistic model

Effect	df	deviance	$p(\chi^2)$
Maximal model	2571	184.87	
Altitude	3433	15.19	<0.001
Intens cultivated pasture	271	9.19	0.027
Grazed area		20.40	<0.001
Medium High Maquis		31.63	<0.001
High Maquis		31.96	<0.001
Null model		373.76	
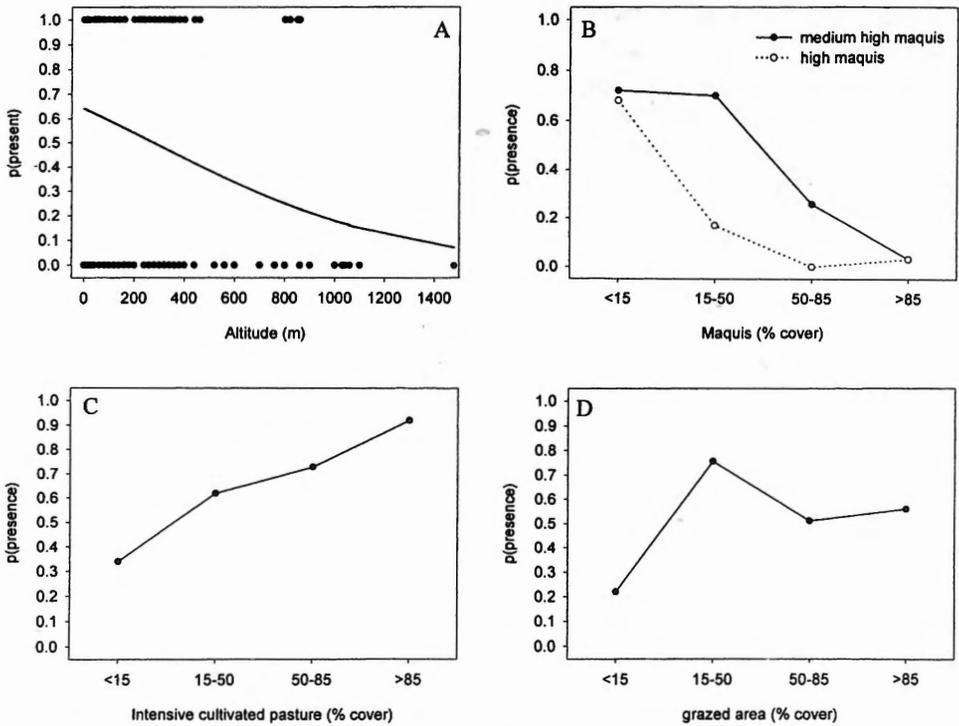
Fig. 2. – Environmental factors affecting the presence of breeding Spotless Starling

DISCUSSION

It appears the predominant factor affecting the presence of reproducing Spotless Starlings is the presence of low vegetation (intensively cultivated pastures without maquis or trees) in the immediate surroundings. It is only at a lower level that some fruit cultures (vine, olive, citrus) seem to predict the occurrence of Spotless Starlings. Some other studies concerning the distribution of the Spotless Starling in Mediterranean areas confirm our results and some contradict them. The Spanish Spotless Starling (MOTIS *et al.*, 1983) was found in every type of culture (olive groves, vineyards, almonds, etc.) and only the predominant presence of trees and scrubs stopped its expansion. Unfortunately no information is given on the period of the Spanish inventories. The period of inventory is important because during the non-breeding period, starlings tend to disperse and use other feeding sources. Our Corsican data confirm the Spanish data in that the absence of medium and high maquis had a positive influence on the presence of the Spotless Starling as did, but less pronounced, the presence of vines, olives and citrus.

There are some reports (CRAMP, 1994) of Spotless Starlings nesting in forests in Moyen Atlas (northwest Africa) making long flights from the forest to open plateaus to collect food. This indicates Spotless Starlings can also reproduce in woody areas, proba-

bly when no other nesting facilities are available. In Corsica however the Spotless Starling was never observed to nest in forests.

We found that sheep and goats appear to promote the presence of the Spotless Starlings. Starlings are often observed following grazers, catching insects disturbed by the animals. Though they sometimes exhibit « oxpecker feeding » (FEARE, 1984), eating ectoparasites while perching on the backs of cows and sheep, starlings more often practise the open bill probing or prying (FEARE, 1984) to capture hidden invertebrates on short grassland. We have to keep in mind that the effect of grazing lasts longer than the actual presence of the animals as grazers move between the different available grassland. The estimate for grazed area was also significantly correlated with the presence of reproducing Spotless Starlings (Fig. 2). Therefore we can state that domestic animals such as sheep and goats indirectly improve the habitat for the Spotless Starlings. By grazing they create the preferred foraging areas of the starling, i.e. short grassland.

It was indicated in former studies that Spotless Starlings are regularly found foraging in vineyards. In our study the presence of vineyards also increased the chance of finding the birds (Table 2). However, pastures often surround vineyards. Our statistical analysis also showed that a habitat is very suitable for the reproduction of the Spotless Starling more through the presence of short grassland rather than through the multitude of fruit cultures (Table 3). As nestlings are mostly fed with invertebrates (PERIS, 1980, RENARD unpubl. data) short grass areas provide the highest amount of prey (FEARE, 1984). STEVENS (1983) found for the European Starling that the birds feeding in orchards were mostly juveniles, probably because this food item is easy to locate and consume. The large flocks of starlings observed in the Corsican orchards, vineyards and olive groves from July onwards are therefore probably mostly composed of early migrating European Starlings and juvenile Spotless Starlings. In autumn and winter it is difficult to estimate the number of Spotless Starlings as they mix with wintering European Starlings when foraging and in dormitories. Only a few birds stay around the breeding area in winter (YEATMAN-BERTHELOT, 1994).

Because the distribution of the Spotless Starling is so dependent of the agricultural land use, it is not surprising that we only found about 2500 breeding pairs, a rather low number compared to the situation in Spain (FERRER et al., 1991). Probably the small-scale agricultural activities in Corsica limit the number of Spotless Starlings on the island and extremely high densities are avoided.

The European Starling is a very successful colonizer. The rapid expansion of the species in North America is well known (43 km per year, MARGALEF, 1974 in FERRER et al., 1991) and has often been connected with the expansion of agricultural areas (FEARE, 1984). The European Starling seems to be more dependent on cereal culture (MOTIS et al., 1983) than is the Spotless Starling. In Spain the expansion of the first is described as a mixed diffusion with short dispersal jumps, whereas the latter shows a continuous forward movement (FERRER et al., 1991). Although in Spain both species prefer areas with low vegetation, the Spotless Starling can be found in dry and high mountain areas where the European Starling remains absent (FERRER et al., 1991). Combining our observations with the Spanish ones it seems the Spotless Starling is more adapted to the Mediterranean climate than is the European Starling, which chooses the « richer » areas. Where both species

are present together, the expansion rates of both drop (FERRER *et al.*, 1991); this observation seem logical as both species compete for food as well as nesting resources. The dry climate and poorly developed cereal culture in Corsica might be the reason the European Starling does not breed in Corsica, while the Spotless Starling can exploit the available resources.

ACKNOWLEDGEMENTS

REFERENCES

ANONYMUS (1989) – Tableau de bord de l'environement Corse. DRAE Ajaccio.

CAMBRONY M. & A. MOTIS (1994) – Statut de l'étourneau unicolore *Sturnus unicolor* en Languedoc-Roussillon en 1993. Alauda **62** (2): 135-140.

CRAMP C. & C. PERRINS (1994) – Birds of the Western Palearctic. Vol. VII. Oxford University Press. (1850 pp).

CRAWLEY M.J. (1993) – GLIM for ecologists. Blackwell Scientific Publications, London (377 pp).

FRANCIS B., M. GREEN & C. PAYNE (1994) – GLIM 4. The statistical system for generalised linear interactive modelling. Clarendon Press, Oxford. (821 pp).

FEARE C. (1984) – The Starling. Oxford University Press (315 pp).

FERRER X., A. MOTIS & S.J. PERIS (1991) – Changes in the breeding range of starlings in the Iberian Peninsula during the last 30 years: competition as a limiting factor. Journ. of Biogeo, **19**: 631-636.

KAYSER Y. & E. ROUSSEAU (1994) – Premières données sur l'étourneau unicolore *Sturnus unicolor* dans le sud de la France. Nos oiseaux, **42** (7): 369-378.

METHA C. & N. PATEL (1995) – StatXact 3 for Windows. Cytel Software Corporation, Cambridge, USA. (788 pp).

MOTIS A., P. MESTRE & A. MARTINEZ (1983) – La colonización y expansión del estornino pinto *Sturnus vulgaris* L. y del estornino negro *Sturnus unicolor* Temm. en Cataluña (NE de la Peninsula Iberica). Misc. Zool., **7**: 131-137.

PATRIMONIO O. (1991) – Inventaire de l'avifaune de Castagniccia (Ampugnani et Orezza) Trav. Sci. Parc Nat. Rég. Corse, **31**: 55-75.

PERIS S.J. (1980) – Biología del estornino negro *Sturnus unicolor*. II. Dieta del pollo. Doaña Acta Vertebrata, **7** (2): 249-260.

PERIS S.J., A. MOTIS & A. MARTINEZ (1987) – La distribución del estornino negro *Sturnus unicolor* Temm. y del estornino pinto *Sturnus vulgaris* L. en la Peninsula Iberica: aumento del area de nidificacion en ambas especies. *Actas VIII Bienal R. Soc. Esp. Historia Natural*: 151-156.

RICE W.R. (1989) – Analyzing tables of statistical tests. *Evol.* Vol. 43 (1): 223-225.

STATSOFT (1994) – STATISTICA for Windows. Statsoft, Inc., Tulsa, USA (3911 pp).

STEVENS J. (1983) – Biotoop keuze en foerageergedrag van adulte en juveniele spreeuwen *Sturnus vulgaris* L. in de Belgische fruitstreek in de kersenperiode. Ph.D thesis K.U.Leuven

THIBAULT J.C. (1983) – Les oiseaux de la Corse. Histoire et répartition aux XIXᵉ et XXᵉ siècles. Gerfau Impression, Paris (225 pp).

YEATMAN L. (1976) – Atlas des oiseaux nicheurs de France. S.O.F. Paris (282 pp)

YEATMAN-BERTHELOT D. (1994) – Nouvel atlas des oiseaux nicheurs de France 85-89. S.O.F. Paris (775 pp).

REPRODUCTION AND SURVIVAL OF THE RICEFIELD RAT *RATTUS ARGENTIVENTER* ON RICE PLANT DIET

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Abstract. Reproduction and survival were studied in caged individuals of the ricefield rat, *Rattus argentiventer,* on a diet of rice plants in various stages of growth. The female rat was reproductively active on diets consisting of rice plant at any stage of growth. However, the reproductive activity of the male rat was significantly influenced by the stage of the rice plant: the male was sexually active while feeding on the generative stages, but almost inactive while feeding on the vegetative stages. The percentage of males with enlarged scrotal testes began to increase on a diet of plants in the panicle primordia initiation stage and reached 100 % by the milky grain stage. The male determines the breeding pattern of this species, and this is directly influenced by the males' food source. Male rats with a scrotal sac containing testes that were larger than 30 mm (measured externally on living rats) were always reproductively active. The survival of both sexes of the ricefield rat also corresponded closely with the growth stage of the rice plant. There were no significant differences in survival values between males and females feeding on different stages of the rice plant. Overall, the milky and ripening grain stages of the rice plant provided the best foods for the reproduction and survival of the ricefield rat.

Key words : Ricefield rat *Rattus argentiventer,* male rat, reproductively active, stage of rice plant, survival.

INTRODUCTION

Periodic eruptions of populations of the ricefield rat, *Rattus argentiventer* (Robinson and Kloss, 1916) cause serious economic losses to agriculture in Indonesia (GEDDES, 1992; HOQUE *et al.*, 1988; MURAKAMI *et al.*, 1990; SOEKARNA *et al.*, 1978). The density of the ricefield rat population fluctuates greatly throughout the year according to the stage of growth of the rice plant (TRISTIANI *et al.*, 1998). A previous field study found that there are two distinct population peaks each year, with each peak occurring two to four weeks after a rice harvest (TRISTIANI *et al.*, 1998). Birth and immigration are the important components that determine the population increase (SEBER, 1973; TRISTIANI *et al.*, 1998).

Numerous authors have investigated the effect of supplemental food or the quality of food on individual reproduction in different small mammal species (BOMFORD, 1987a, 1987b; TANN *et al.*, 1991; DUQUETTE & MILLAR, 1995). The reproductive performance of small mammals is affected by either the quality or the abundance of available food, and also by temperature and social factors (SADLEIR, 1969). Results from previous studies sug-

gest that the nutritional quality of the rice plant also has an important influence on the rate of population increase in the ricefield rats (TRISTIANI *et al.,* 1998).

Although the general biology, breeding and control of the ricefield rat were studied in Malaysia by HARRISON (1951), LAM (1980) and BUCKLE *et al.* (1985), little is known of the population dynamics of this species in relation to the developmental stages of the rice plant. Specifically, there is little research that clearly examines the relationship between the reproduction and survival of rat populations and the stage of growth of the rice plant.

This study has two aims. The first is to look for a relationship between the reproduction and survival of the ricefield rat, and its diet on different stages of the growing rice plant. Secondly, we hoped to identify the growth stages that limit the reproduction and survival of these rats.

MATERIAL AND METHODS

The response of ricefield rats to diets consisting of rice plants at different growth stages was studied under laboratory conditions at Jatisari Field Station, West Java, Indonesia. Rats were live-trapped in adjacent rice fields and kept in separate cages measuring 460 x 350 x 190 mm. All captured rats were sorted and classified as adult males (=110 g), adult females (= 60 g), sub-adult males (41-109 g), sub adult females (41-59 g) and juveniles (= 40 g) on the basis of weight.

The temperature in the laboratory ranged from 22° C to 33° C (mean = 26.82° C) and the relative humidity ranged from 45% to 91% (mean = 73.13%). All experiments were carried out simultaneously in the laboratory, therefore conditions were the same across treatments.

During an initial twenty-four day quarantine period, the rats were fed unhusked rice and water was provided *ad libitum.* At the end of this period, all rats were sorted and only pre-pubertal rats which had not yet reproduced were used in this study.

After a 24-hour starvation period (water was continually available), the rats were provided with fresh rice plants in various stages of growth. Eight stages of the rice plant were tested:

Vegetative

1) seedling
2) three weeks after transplantation (3 wat)
3) maximum tillering

Generative

4) initiation of the panicle primordia (OTA, 1990)
5) booting
6) flowering
7) milky
8) ripening

In addition, a ninth diet, consisting of a combination of grass, weeds and grasshoppers, was also tested. Three common weeds/grasses of rice paddies (*Echinochloa crusgalli, Cyperus difformis* and *Cyperus serotinus*) were used in this last diet.

The plants were replaced each afternoon and the quantity of vegetable material remaining was measured the following morning. The rats were weighed twice daily. Groups of 30 rats each were fed separate, single stage material from the cropping cycle for a two month period or until the death of the rat. Two commonly cultivated varieties of rice (IR64 and Cisadane) were used. Out of a total 510 rats, there were 255 males and 255 females.

The following data were recorded for each rat: weight, sex, position of testes (abdominal or scrotal testes) for males, the presence or absence of vagina perforate and size of nipple for females. At death or at the end of the experiment, the size, weight, and condition of the testes and caudal epididymis were determined by necropsy. Males with enlarged scrotal testes and swollen, whitish epididymis were considered to be reproductively active. Females were classified as reproductively active if they had a perforate vagina and or large nipples.

RESULTS

Only one of the sixty males fed a vegetative stage diet (Cisadane variety) was found to be reproductively active, while all the males fed a milky or ripening stage diet became reproductively active five days after being put on this diet. An increasing proportion of the males fed on intermediate maturation stage diets of either variety of rice became reproductively active. However, rats feeding exclusively on the Cisadane variety developed more rapidly (the difference with diets in the booting stage was statistically significant ($P>0.05$)) (Fig. 1). None of the males became reproductively active on the grass/weed/
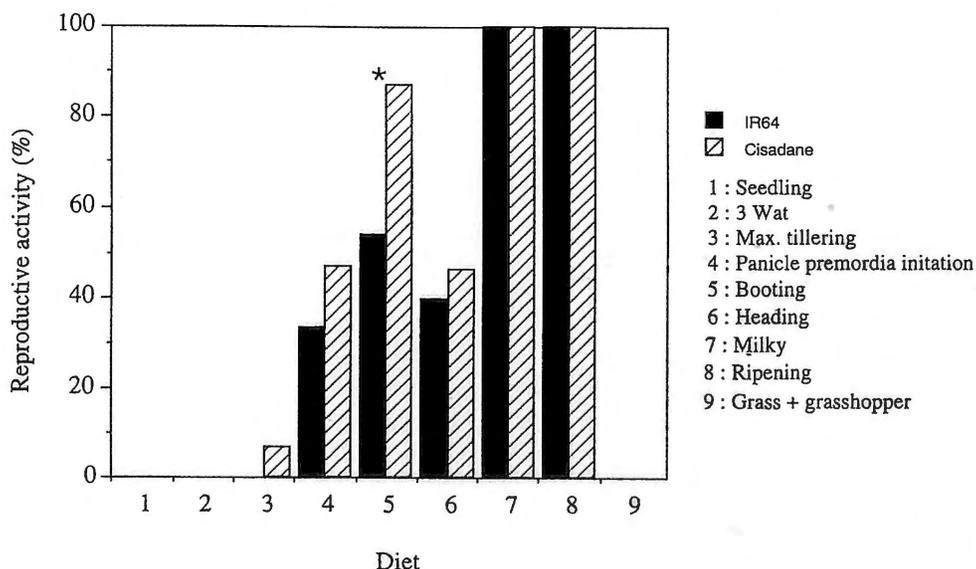
Fig. 1. – The percentage of male rats (*Rattus argentiventer*) that were reproductively active during various stages of the rice plant. [Asterisk denotes a significant difference between IR64 and Cisadane varieties (d.f.=1, $P>0.05$)].

grasshopper diet. In contrast, all the females became reproductively active (perforate vagina) on diets consisting of either variety of rice at any stage, as well as on the grass/weed/grasshopper diet.

Among the reproductive male rats, there was a further relationship between the relative development of the testes and the length of the scrotum containing descended testes. When the scrotal sac containing testes was larger than 30 mm (measured externally on living rats) the males were reproductively active (Fig. 2).

scrotal sac length in mm
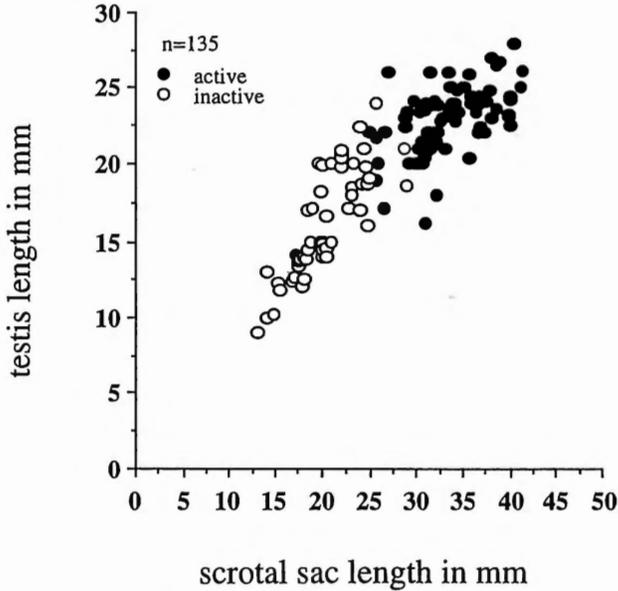
Fig. 2. – The relationship between index of testis (p-t index) to the reproductive state of the testis. (P-T index is the size of the scrotal sac with testis inside measured on the live rat from outside).

Rat survival was also affected by the stage of rice growth given. All the rats fed milky and ripening stages diets survived the two-month test (Table 1). Average survival times on diets of both the IR64 and Cisadane varieties in the booting stage were long, an average of fifteen and twenty days, respectively. Survival dropped sharply, to an average of five days, on a diet of plants in the flowering stage. The grass/weed/ grasshopper diet resulted in the shortest survival times, an average of four days. Although there were significant differences in survival due to differences in diet, there were no significant differences (at a 5% level) between the survival of males and females on the various diets (Table 1).

Relative daily food consumption was almost the same (not significant at a 5% level) from the early growth stage of rice plant to the flowering stage, but then decreased after the milky stage (Fig. 3). From the early growth stage of the rice plant to the flowering

stage, there were no significant differences (at a 5% level) between the quantities of the two varieties consumed nor between quantities of each growth stage consumed.

TABLE 1

Rice plant variety	Diet (Stage of rice plant)	Male[a] (mean ± 95% CL)	Female[a] (mean ± 95% CL)
IR64	Nursery bed	10.3 ± 2.63	10.1 ± 1.95[ns]
	Tillering	9.0 ± 2.03	8.9 ± 1.91[ns]
	Max. tillering	8.8 ± 2.15	8.6 ± 1.80[ns]
	Panicle premordia	10.3 ± 2.10	10.2 ± 1.69[ns]
	Booting	15.2 ± 4.28	15.0 ± 4.03[ns]
	Flowering	5.3 ± 2.38	5.3 ± 1.47[ns]
Cisadane	Nursery bed	11.9 ± 2.18	11.3 ± 1.56[ns]
	Tillering	9.5 ± 2.22	9.5 ± 1.91[ns]
	Max. tillering	11.8 ± 3.59	11.1 ± 2.94[ns]
	Panicle premordia	12.0 ± 3.69	11.9 ± 4.16[ns]
	Booting	20.1 ± 2.79	20.0 ± 2.49[ns]
	Flowering	5.5 ± 2.87	5.3 ± 2.29[ns]
	Grass/weed + grasshopper	4.4 ± 2.21	4.3 ± 2.00[ns]

[a] n=15 for each experimental class
ns: Non significant differences between males and females at 5% level as determined by Chi-square analysis
Note: In the milky and ripening stages diet, all rats were alive at the end of the experiment.
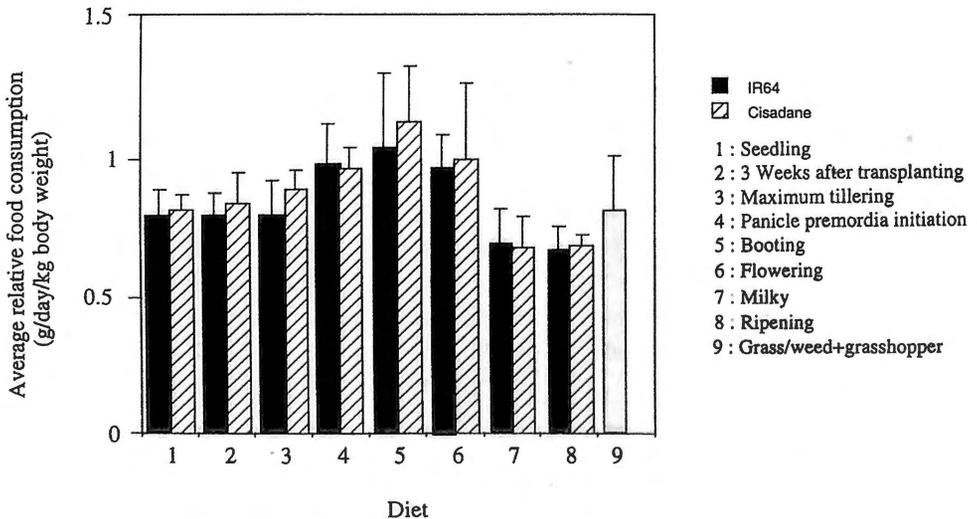
Fig. 3. – Average relative food consumption of the ricefield rat *R. argentiventer* for different stages of the rice plant [the vertical line is Standard Error (SE)].

DISCUSSION

The reproductive rate and population density of small rodents depend on the abundance and/or quality of food (TAITT, 1981; ANDERSON & JONASSON, 1986; DESY & BATZLI, 1989; LEIRS et. al., 1994). The reproductive activity of male R. argentiventer was influenced by rice plant development; the males were only sexually active when feeding on rice plants in the generative stages of growth. The percentage of males with enlarged scrotal testes increased during the initiation of the panicle primordia, booting, and heading stages, and reached 100% by the milky stage. Some substances initiating male reproductive activity may be present in the the generative stage of the rice plant. Several small rodent species show similar sensitivity to their diets on green plants (PINTER & NEGUS, 1965; NEGUS & PINTER, 1966; BOMFORD, 1987a, 1987b). NEGUS & PINTER (1966) reported that adding fat extracts from wheat sprouts to laboratory diets increased the reproductive performance of Microtus montanus. DESY & BATZLI (1989) in their study of prairie vole reported that supplemental high-quality food significantly increased body growth rates, the proportion of adults in the population, reproductive activity, recruitment, and the population density of prairie vole.

This study found that when the length of the scrotum of ricefield rats containing descended testes was larger than 30 mm, the male was reproductively active. Reproductively active males had enlarged scrotal testes and swollen, whitish epididymides. The position of testes is a relatively accurate predictor of the reproductive status of males (McCRAVY & ROOSE, 1992).

All the females were observed to become reproductively active on diets of plants from either variety of rice at any stage of growth, as well as on the grass/weed/grasshopper diet. Female rats almost always mature sooner than male rats. The results of a laboratory rearing study (MURAKAMI, unpublished) found that the earliest maturation of a female (perforate vagina) occurred at 28 days of age when the rat weighed 30 g. In contrast, the earliest maturation of a male rat was 59 days of age at a weight of 90 g. Females are ready to breed anytime after they mature (when they develop perforate vaginas) and remain reproductively active continuously (MURAKAMI, unpublished). In this study females were classified as reproductively active if they had a perforate vaginas. In this study we did not examine female pregnancy or lactation. However, it is known that females with perforate vaginas have a higher possibility of becoming pregnant if they mate with active reproductive males (MURAKAMI, unpublished). FIRQUET et. al. (1996) and KREBS (1966) noted that females with perforate vaginas are usually in breeding condition.

Since female ricefield rats mature sexually regardless of stage of rice plant, it is the reproductive condition of males that determines the breeding pattern in this species, and this is directly influenced by the males' food source.

When only grass and grasshoppers were eaten, male ricefield rats did not become sexually active and survival was low. Although laboratory experiments (SOEKARNA et al., 1978) have found that rats fed exclusively on weeds, green rice stalks, crabs and snails had low survival (4-5 days), we did not attempt to determine why supplementing the diet did not enhance the reproductive activity of the ricefield rat R. argentiventer.

The results of our study show that the seasonal breeding of *R. argentiventer* coincides with the maturation of the rice crop. We conclude that associated nutritional factors are essential for reproduction. Similarly, TAYLOR & GREEN (1976) observed that seasonal patterns in the diet are very closely linked to reproduction of African rodents. MERGES (1972) showed that the pattern of reproduction of *Rattus rattus mindanensis* in the Philippines was very closely related to the growth of the rice crop.

The survival of both sexes of the ricefield rat was also closely related to the growth stages of the rice plant. Stages prior to the initiation of the panicle primordia stage were unfavorable food sources. Although survival increased with a diet of booting plants, there was a sharp drop at the flowering followed by an increase in subsequent stages. In their experiments, SOEKARNA *et al.* (1978) observed extended survival (up to 3 months) in rats fed exclusively on the green tops of younger plants.

Our study found that relative daily food consumption was almost the same from the early growth stage of rice plant to the flowering stage, but then decreased after the milky stage. It is presumed that the quality of the food increased from the milky to the ripening stage. BOMFORD (1987c) reported that milk-ripe seeds of rice plant were full-sized or nearly so, and their endosperm contains starch granules, they are still green and if squeezed exude a milky juice. Chemicals in milk-ripe seeds might signal the arrival of good food supplies to a granivore, just as chemicals in sprouts apparently give such a signal to herbivores (PINTER & NEGUS, 1965; NEGUS & BERGER, 1977; SANDERS *et. al.*, 1981).

While the ricefield rats were fed on rice plants in all stages of growth, the palatability and presumably the nutritive value differed between the stages.

Viewing Fig.1 and Table 1, we can note that, for the male rats, during the generative stage of rice plant, the longer they live, the more sexually active they become (Fig. 4).
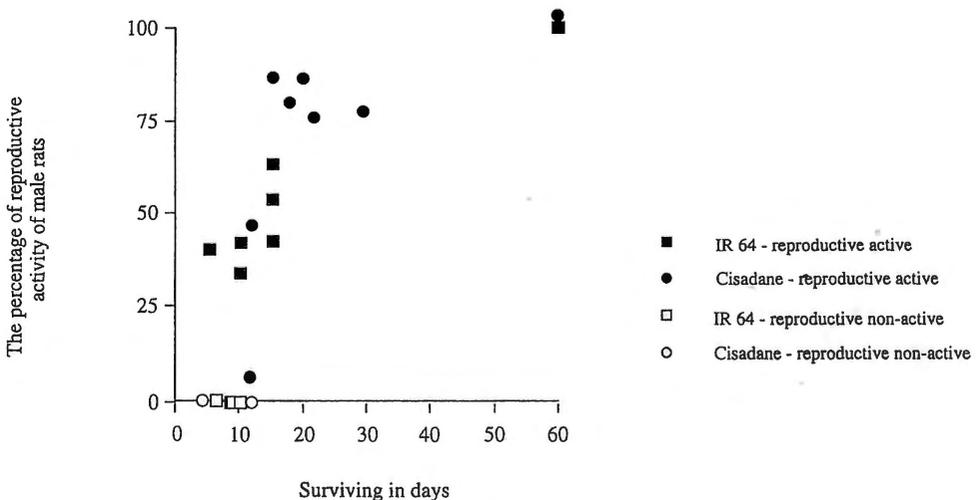
Fig. 4. – The relationship between the percentage of sexually active rats and survival of male rats during the generative stage of rice plant.

By observing the reproduction and survival of the ricefield rats under laboratory control diets of rice plants, we are able to show that the stage of the rice plants affects the reproductive condition and survival of the rats.

ACKNOWLEDGEMENTS

REFERENCES

ANDERSON, M. & S. JONASSON (1986) – Rodent cycles in relation to food resources on an alpine heath. *Oikos.*, **46**: 93-106.

BUCKLE, A.P., Y.C. YONG & H.A. RAHMAN (1985) – Damage by rats to rice in South-east Asia with special reference to an integrated management scheme proposed for Peninsular Malaysia. *Acta Zool. Fennica*, **173**: 139-144.

BOMFORD, M. (1987a) – Food and reproduction of wild house mice I. Diet and breeding seasons in various Habitats on irrigated cereal farms in New South Wales. *Aust. Wildl. Res.*, **14**:183-196.

BOMFORD, M. (1987b) – Food and reproduction of wild house mice II. A field experiment to examine the effect of food availability and food quality on breeding in spring. *Aust. Wildl. Res.*, **14**:197-206.

BOMFORD, M. (1987c) – Food and reproduction of wild house mice III. Experiments on the breeding performance of caged house mice fed rice-based diets. *Aust. Wildl. Res.*, **14**: 207-218.

DUQUETTE, L.S. & J.S. MILLAR (1995) – Reproductive response of a tropical mouse, *Peromyseus mexicanus*, to changes in food availability. *J. Mammal.*, **76**: 596-602.

DESY, E.A. & G.O. BATZLI (1989) - Effects of food availability and predation on prairie vole demography: a filed experiment. *Ecology*, **70**: 411-421.

FIRQUET, E., H. LEIRS & G. BRONNER (1996) – Germinating grasses and reproductive seasonality of Mastomys-species (Rodentia, Muridae). *Mammalia*, **60**: 775-779.

GEDDES, A.M.W. (1992) – *The relative importance of pre-harvest crop pest in Indonesia*. Chatman, UK., Natural Resources Institute Bulletin, **47**: 70 pp.

HARRISON, J.L. (1951) – Reproduction in rats of the subgenus *Rattus*. *Proc. Zool. Soc. Lond.*, **121**: 673-694.

HOQUE, M.M., F.F. SANCHEZ & E.A. BENIGNO (1988) – Rodent problems in selected countries of Southeast Asia and Island in the Pasific. In: *Rodent Pest Management*. (Ed. I. PRAKASH), CRC Boca Raton: 85-99.

KREBS, C.J. (1966) – Demographic changes in fluctuating populations of *Microtus californicus*. *Ecol. Monogr.*, **36**: 239-272.

LAM, Y.M. (1980) – Reproductive behavior of the rice field rat, *Rattus argentiventer* and implication for its control. In: *Proceeding of the National Rice Conference*, Malaysia: 243-257.

LEIRS, H., R. VERHAGEN & W. VERHEYEN (1994) – The basis of reproductive seasonality in Mastomys rats (*Rodentia: Muridae*) in Tanzania. *J.Trop.Ecol.*, **10**: 55–65.

MCCRAVY, K.W. & R.K. ROSE (1992) – An Analysis of External Features as Predictors of Reproductive Status in Small Mammals. *J. Mammal.*, **73**: 151-159.

MERGES, B.E. (1972) – *Reproduction and seasonal abundance of the ricefield rat (Rattus rattus mindanensis* Mearns) *at Siniloan, Laguna*. Unpublished MSc. Thesis, University of the Philippines, Los Banos, 43 pp.

MURAKAMI, O., J. PRIYONO & H. TRISTIANI (1990) – Population management of the ricefield rat in Indonesia. In: *Rodents and Rice, report and proceedings of an expert panel meeting on rice roden control*. (Ed. Gr. Quick), Manila, Philippines, International Rice Research Institute: 49-54.

NEGUS, N.C. & A.J. PINTER (1966) – Reproductive responses of *Microtus montanus* to plant and plant extracs in the diet. *J. Mammal.*, **47**: 596-601.

NEGUS, N.C. & P.J. BERGER (1977) – Experimental triggering of reproduction in a natural population of *Microtus montanus*. *Science (Wash.D.C.)*, **196**: 1230-1231

OTA, Y. (1990) – *Physiology of Rice Plant. Rice Pest Management in Japan (I)*. Japan International Cooperation Agency Hyogo International Center, Japan : 23-54.

PINTER, A.J. & N.C. NEGUS (1965) – Effects of Nutritional and photoperiod on reproductive physiology of *Microtus montanus*. *Am. J. Physiol.*, **208**: 633-638.

SADLEIRS, R.M.F.S. (1969) – *The Ecology of Reproduction in Wild and Domestic Mammals*. Methuen, London. 321 pp.

SANDERS, E.H., P.J. GARDNER & N.C. NEGUS (1981) – 6-methoxybenzoxazolinone: a plant derivative that stimulates reproductive activity in *Microtus montanus*. *Science (Wash.D.C.)*, **214**: 67-69.

SEBER, G.A.F. (1973) – *The estimation of animal abundance and related parameters*. Griffin, London. 506 pp.

SOEKARNA, D., S. PARTOATMODJO, S. WIRJOSUHARDJO & BOEADI. (1978) – Problems and Management of Small Mammals in Indonesia with Special Reference to Rats. In: *Symposium on small mammal problems and control*. Los Banos, Philippines: 1-31.

TAITT, M.J. (1981) – The effect of extra food on small rodent populations: I. Deermice (*Peromyscus maniculatus*). *J.Anim. Ecol.*, **50**: 111-124.

TANN, C.R, G.R. SINGLETON & B.J. GOMAN (1991) – Diet of the house mouse, *Mus domesticus*, in the Mallee Wheatlands of North-western Victoria. *Wild. Res.*, **18**: 1-12.

TAYLOR, K.D & M.G. GREEN (1976) – The influence of rainfall on diet and reproduction in four African rodent species. *J.Zool., Lond.*, **180**: 367-389.

TRISTIANI, H., J. PRIYONO & O. MURAKAMI (1998) – Seasonal changes in the population density and reproduction of the ricefield rat, *Rattus argentiventer* (Rodentia: Muridae), in west Java. *Mammalia*, **62**: 227-239.

SPERMATOGENESIS OF *HAPLOPHARYNX ROSTRATUS* (PLATYHELMINTHES, HAPLOPHARYNGIDA)

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Abstract. During spermatogenesis, nuclei of spermatids are at first large with an irregular outline, containing chromatin granules; then condense to become round containing dense chromatin; then elongate containing loose fibrillar chromatin. Golgi bodies produce dense granules. Immature sperm in the testis are amoeboid, aflagellate, with numerous peripheral microtubules. Mature sperm in the sperm duct have a nucleated part with a narrow layer of cytoplasm drawn out into two flanges, and a thicker non-nucleated part. Peripheral microtubules and dense thickenings of the plasma membrane are present in both parts. Two symmetrically arranged blunt bristle-like structures, apparently modified large granules that do not reach the surface, are located in the nucleated part. Sperm structure supports the view that Haplopharyngida and Macrostomida are closely related.

Key words : Platyhelminthes, Haplopharyngida, *Haplopharynx rostratus*, sperm, spermatogenesis, ultrastructure, phylogeny

INTRODUCTION

The Haplopharyngida are usually considered a taxon separate from but close to the Macrostomida (*e.g.*, CANNON, 1986). EHLERS (1985) includes them in the Macrostomida. DOE (1986) found a matrix syncytium of the copulatory stylet in both taxa supporting their monophyly. Not a single electron-microscopic study of sperm and spermatogenesis of a haplopharyngid has been made (WATSON & ROHDE, 1995), whereas several such studies of macrostomids have been published (BEDINI & PAPI, 1970; NEWTON, 1980; ROHDE & WATSON, 1991; ROHDE & FAUBEL, 1997 a, b). In this paper we describe the ultrastructure of sperm and spermatogenesis of *Haplopharynx rostratus* MEIXNER, 1938 with the aim of contributing to a better understanding of the phylogeny of the Platyhelminthes in general and of the Haplopharyngida in particular.

MATERIAL AND METHODS

One specimen of *Haplopharynx rostratus* was collected from the beach near the old Research Station on the Island of Sylt, North Sea, and immediately fixed in 3 % glutaraldehyde

in 0.1M sodium cacodylate buffer (pH 7.2) for about one week at room temperature. It was washed for 40 minutes at 4°C in 0.1 sodium-cacodylate buffer (pH 7.2) made up with seawater, postfixed for 1 hr in 1 % OsO_4 in the same buffer and dehydrated in a graded series of ethanol. It was embedded in Spurr's resin. Serial longitudinal sections were stained with uranyl acetate and lead citrate and examined under a JEOL 1200EX electron microscope at 60 kV.

RESULTS

Primary spermatocytes, clearly characterised by their synaptonemal complexes (Fig. 1 A, B) have large nuclei and light cytoplasm. Cells with much more electron-dense nucleoplasm and distinctly aggregated chromatin, and with dense cytoplasm containing much endoplasmic reticulum (Fig. 1 A) are interpreted as spermatogonia, although the possibility cannot be excluded that they are secondary spermatocytes. The second alternative is not unlikely in view of the well developed endoplasmic reticulum, which is unusual for spermatogonia. Spermatids contain Golgi complexes producing many vacuoles (Fig. 1 C, D), their nuclei are at first large with light nucleoplasm containing many chromatin granules, but later condense and become round, containing many small electronlucent areas (Fig. 2 A-C). Mitochondria aggregate around the nucleus (Fig. 2 B). Lack of membranes between some nuclei indicates that spermatids form syncytial clusters. At an even later stage of spermiogenesis, the chromatin becomes loosely fibrillar and the cells and nuclei elongate (Fig. 2 D). Sperm in the testis are amoeboid, with many deep invaginations and lobe-like processes (Figs 3 A-C, 4 A). They contain numerous electron-dense granules of a range of sizes (Fig. 5), many of them observed to be lined by distinct membranes (Figs 3 A-E, 4 A). They also contain mitochondria (Figs 3 E, 4 A), a nucleus with fibrillar chromatin (Fig. 3 C, D), aggregations of dense material and more or less electronlucent vacuoles (Fig. 3 C), and their surface has a densely packed row of peripheral microtubules below the plasma membrane (Figs 3 B, E, 4 A). The plasma membrane has many dense thickenings, some of which at least are artefacts (*e.g.*, Fig. 3 E).

Mature or maturing sperm in the sperm duct have a narrow part containing the dense nucleus with very small lucent spaces, surrounded by a thin layer of cytoplasm with mitochondria and dense granules, and this narrow part is drawn out into two flanges (Figs 4 C, 6 A-E). A row of microtubules is found below the surface membrane (Figs 4 C, 6 A-D). Some microtubules are present below the peripheral row (Figs 4 A, 6 B). The non-nucleated parts of the sperm are much larger in cross-section, and they contain a much greater number of dense granules of various sizes; the surface membrane also has a row of microtubules (Fig. 4 B-D). Two symmetrically located curved and tapering structures («bristles») that do not reach the surface and apparently are modified granules, are located in the nucleated part of the sperm close to the nucleus (i.e., not at the tips of the flanges), in the region close to the beginning of the flanges (Figs 6 B, 7 A, B). Dense structures seen once at the tip of the flange (Fig. 6 B) are of unknown nature but may represent a disintegrating granule.

The wall of the sperm duct is formed by cells in contact by septate junctions. Cilia are not densely packed but occur all around the duct (Fig. 4 C, D).

Diagrams of mature sperm are given in Fig. 8.
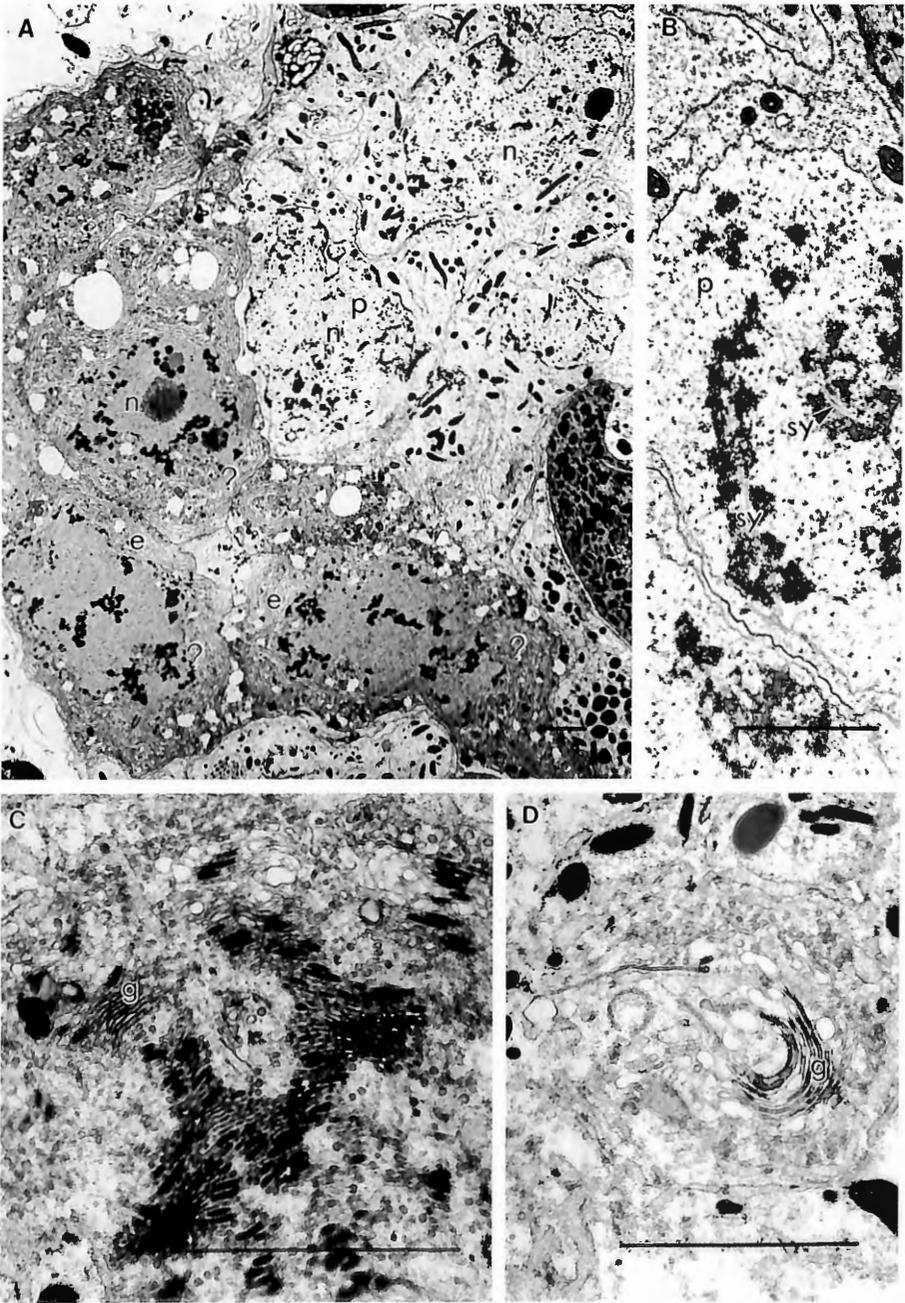
Fig. 1. A. – Section through testis with primary spermatocytes (?) on the right (p), and spermatogonia or secondary spermatocytes on the left. Also note endoplasmic reticulum (e) and nuclei (n). B. Synaptonemal complexes (sy) in primary spermatocytes (p). C, D. Golgi complexes (g) in spermatids. Scale bars 2μm.
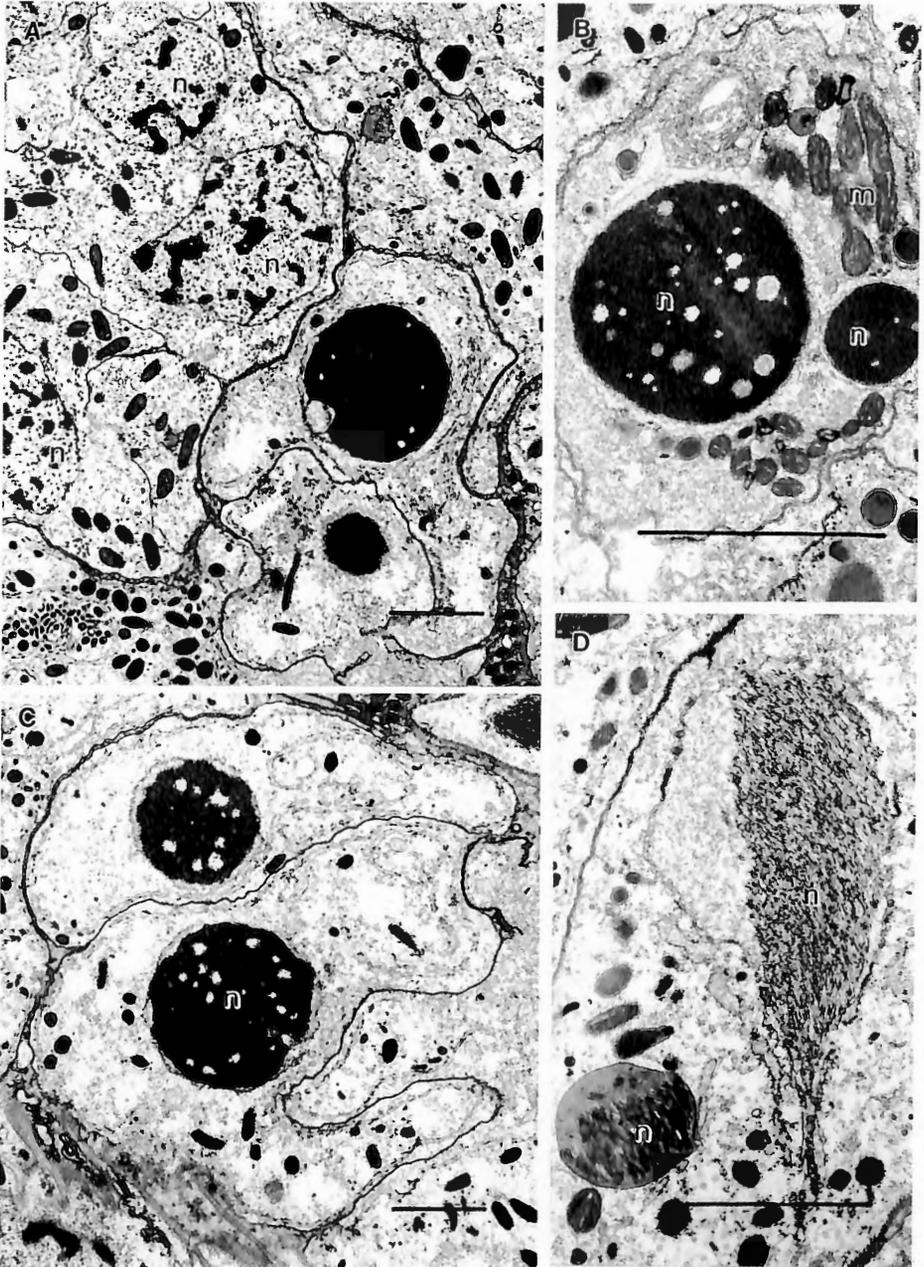
Fig. 2. A. – Section through testis with spermatids containing uncondensed nuclei (n). Note rounded compact nuclei of spermatids on the right. B. Spermatids with rounded nuclei (n) and many mitochondria (m) near them. Note lack of membrane between nuclei. C. Two spermatids with rounded nuclei (n). Note dense chromatin with some electron-lucent areas in B and C. D. Slightly later stage of spermatid. Note nuclei (n) with loose-fibrillar configuration, elongating on right. Scale bars 10µm (A), 2µm (B-D).
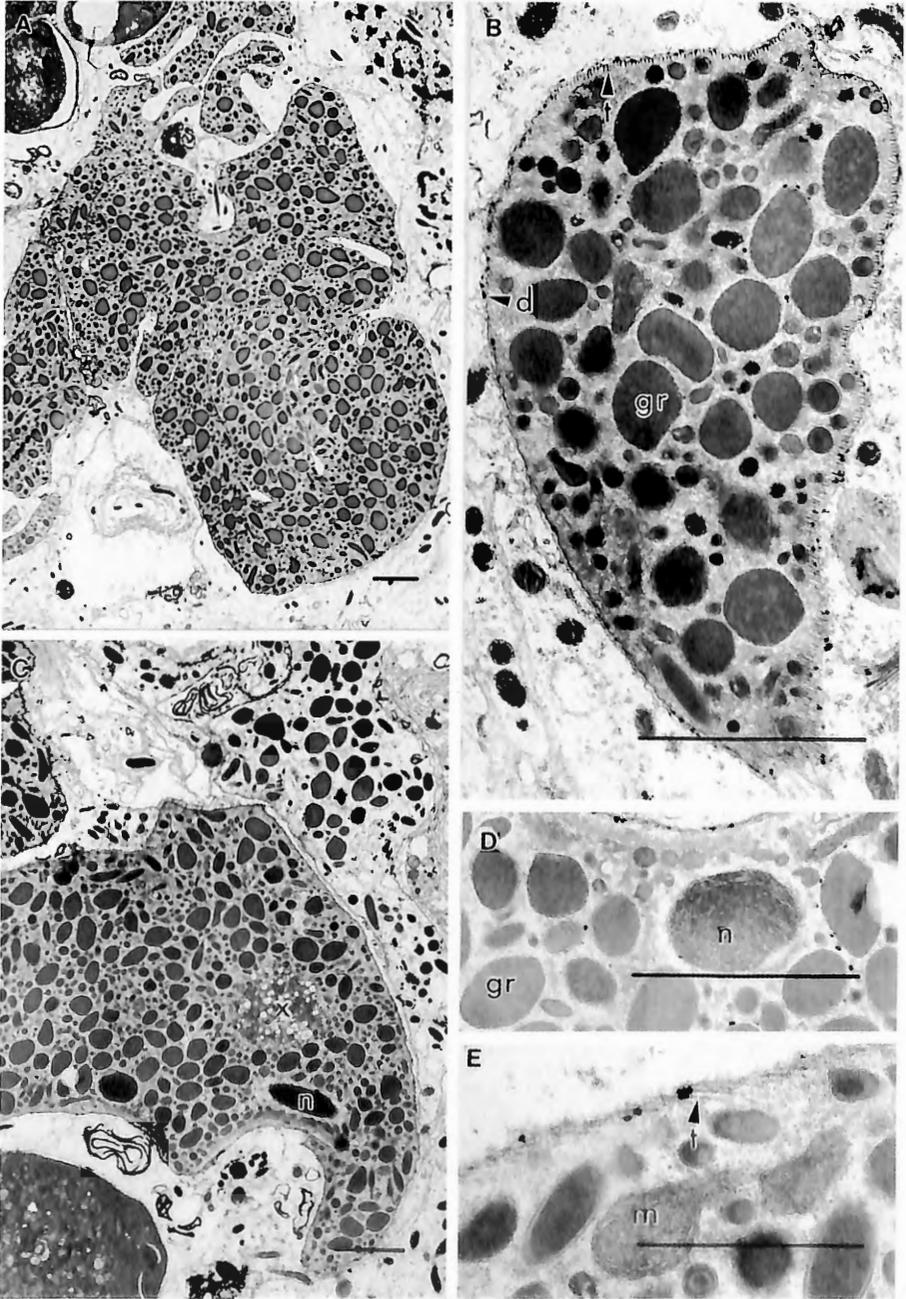
Fig. 3. – Sperm in testis. Note amoeboid shape (A-C), numerous electron-dense granules (gr) of different sizes, some with distinct membranes, nucleus (n), and mitochondria (m). Also note the vesicular regions (x) in C, the peripheral microtubules (t) and dense thickenings (d) (some of them apparently artefacts)... Scale bars 2μm (A-D), 1μm (E).
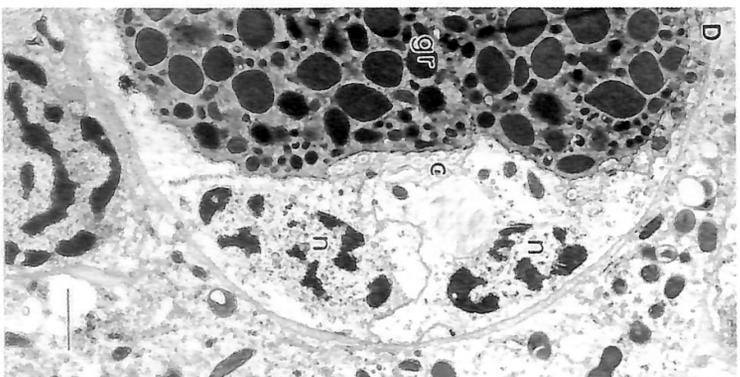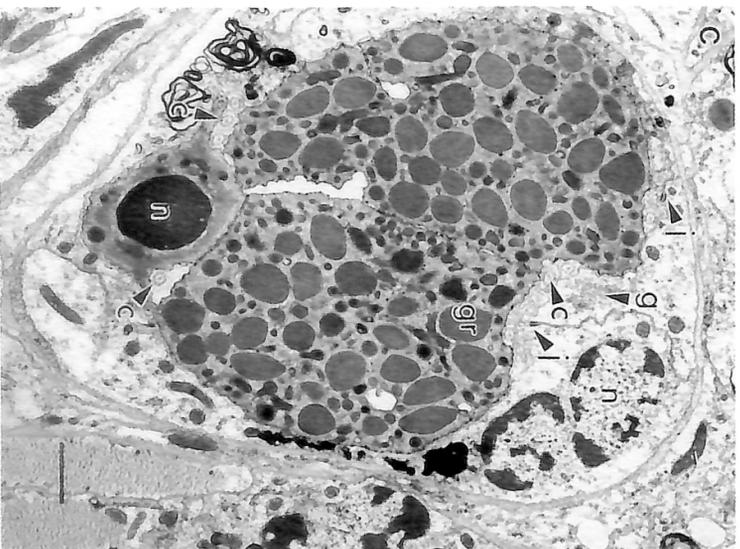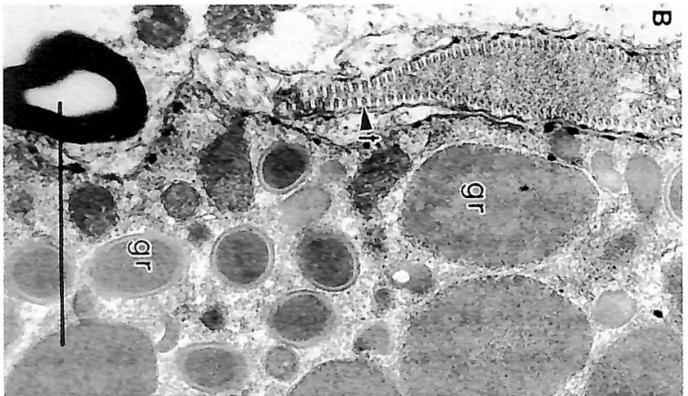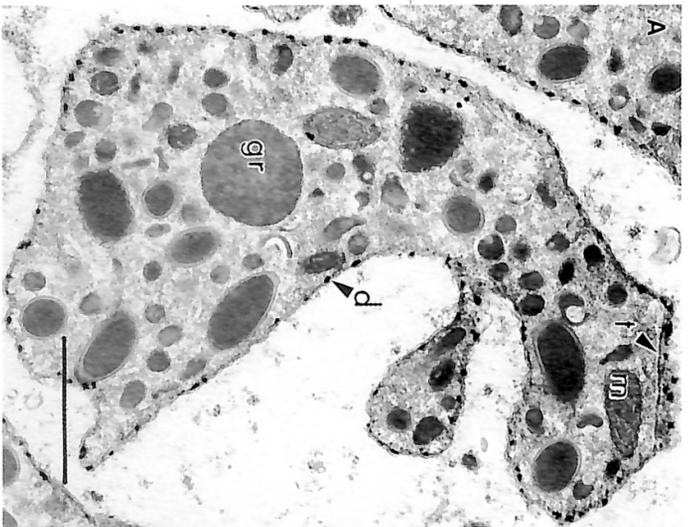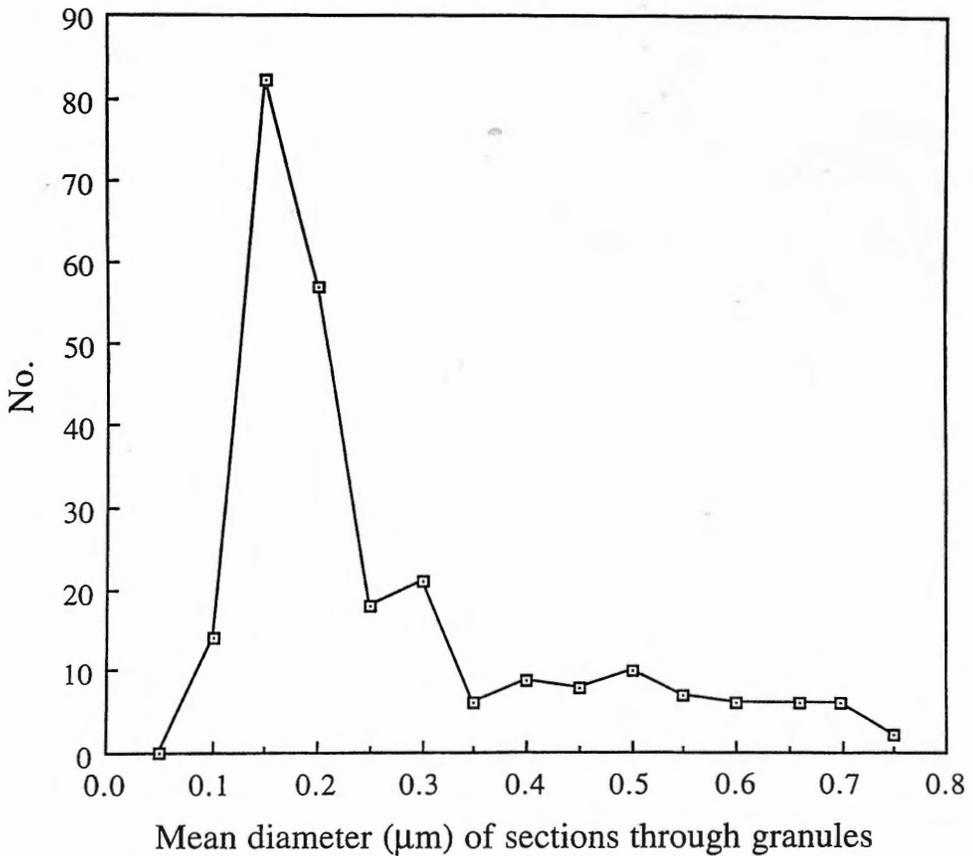
Fig. 5 . – Frequency distribution of mean diameters of sections through granules in sperm from the testis.

Legend to the figure (see page 182)

Fig. 4. – A. Sperm in testis. Note peripheral microtubules (t) and dense thickenings (d), mitochondria (m) and granules (gr) of different sizes, some with distinct membranes. B. Sperm in sperm duct. Note marginal section through sperm on left, showing densely packed microtubules (t). C, D. Sperm in sperm duct. Note dense-fibrillar nucleus (n) with some lucent spaces, granules (gr), mitochondria (m), peripheral microtubules (t) and dense thickenings (d). Also note that nucleated part of sperm has only a thin layer of cytoplasm drawn out into two flanges, the non-nucleated parts are thicker and packed with granules. The wall of the sperm duct consists of cells in contact by septate junctions (j); cilia (c) are not densely packed but occur all around the duct. Note nucleus (n) of sperm duct in C, and two nuclei (n) in D. Scale bars 1 µm.
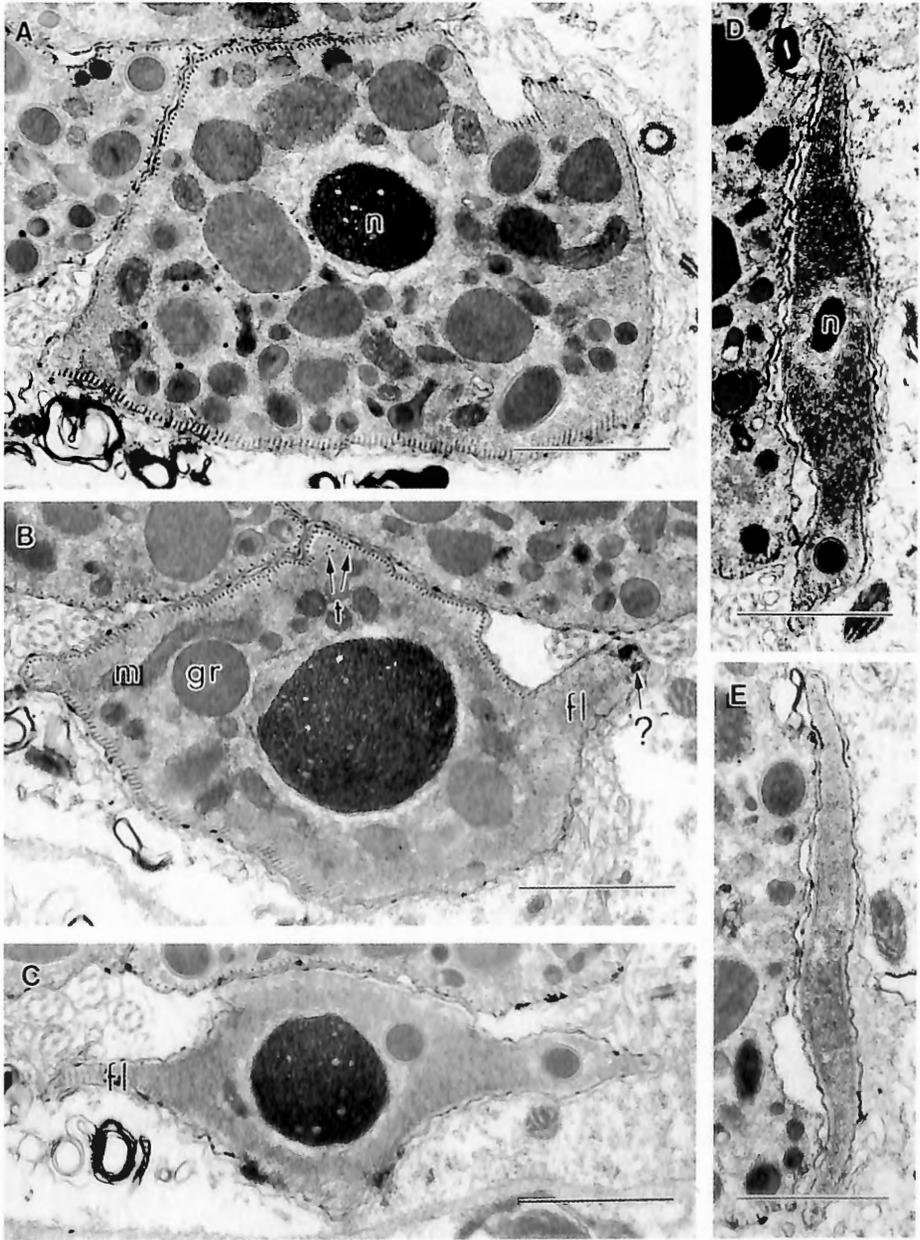
Fig. 6. – Transverse sections through nucleated part of one spermatozoon in sperm duct. A. Section through wide part with many granules and end of nucleus (n). B. Section through part at beginning of flanges (fl), with dense structures of unknown nature (?). Note peripheral row of microtubules (t), and some microtubules below the peripheral row, granules (gr) and mitochondrion (m). C. Section through part with flanges (fl). D. Section through part near the end of nucleus (n). E. Section through tip without nucleus. Scale bar 1μm.
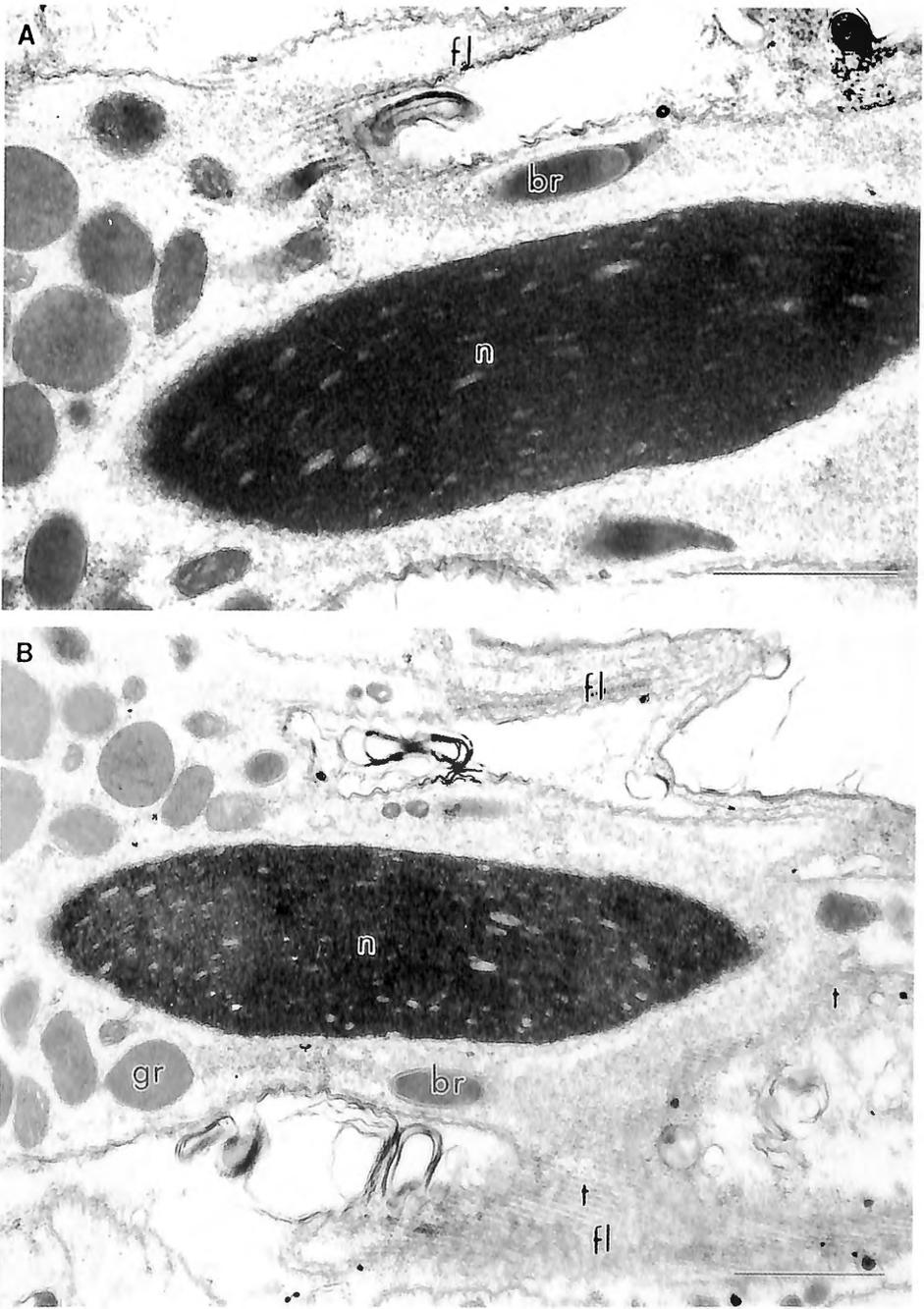
Fig. 7. – Longitudinal sections through sperm at level of beginning of flanges (fl), with nucleus (n), granules (gr), and two symmetrically located «bristles» (br). Also note longitudinal sections through peripheral microtubules. Scale bar 1 μm.
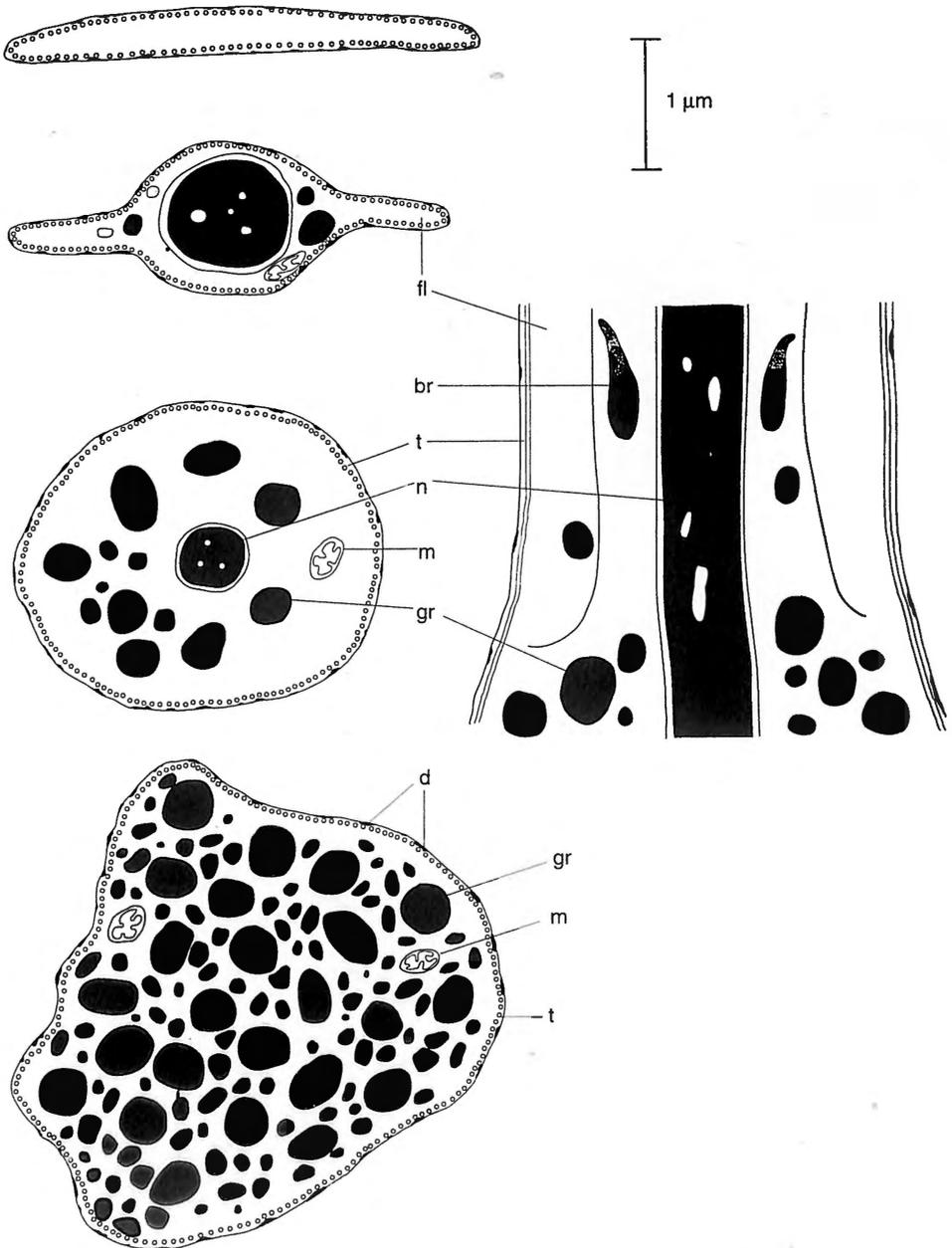
Fig. 8. – Diagrams of cross-sections through sperm (left), and of longitudinal section in the region of the flanges and bristles. Note «bristles» (br), dense thickenings (d), flanges (fl), granules (gr), mitochondria (m), nucleus (n), and microtubules (t).

DISCUSSION

Spermatozoa of *Haplopharynx rostratus* resemble all macrostomids examined in the lack of flagella, and most macrostomids in the presence of bristle-like structures, which are absent in *Paromalostomum fusculum*. They are apparently modified granules, which do not reach the surface of sperm in *Haplopharynx rostratum*. Differences between the two taxa are the number and kind of symmetry of the peripheral microtubules. In *Macrostomum tuba* Graff, 1882 and *M. pusillum* Ax, 1954 (Macrostomatidae), micro-tubules are arranged in two contralateral rows (ROHDE & WATSON, 1991; ROHDE & FAUBEL, 1997b). In *Paromalastomum fusculum* Ax, 1952 (Dolichomacrostamidae), the number of microtubules in each row is small (usually four), and a conclusion concerning the kind of symmetry is not possible (ROHDE & FAUBEL, 1997a). Furthermore, centrioles were found during spermiogenesis, apparently resorbed by the cytoplasm during sperm formation (ROHDE & FAUBEL, 1997a).

Sperm of *Haplopharynx rostratum* differ from those of the macrostomids in the much larger number of peripheral microtubules. Immature sperm from the testis differ from those of macrostomids in the distinctly amoeboid shape. Other aspects of spermiogenesis have no peculiar features.

Altogether, similarities of sperm structure in *Haplopharynx rostratum* and macrosto-mids support the view that haplopharyngids and macrostomids are closely related, although one of the synapomorphies, lack of flagella in mature sperm, is a purely negative characteristic due to secondary reduction (as indicated by the presence of centrioles dur-ing certain stages of spermiogenesis in *P. fusculum*), also found in some other platy-helminths (WATSON & ROHDE, 1995). Large bristles are present in *M. tuba* (ROHDE & WATSON, 1991), and rudimentary ones reaching the surface of sperm in *M. pusillum* (ROHDE & FAUBEL, 1997b). *P. fusculum* lacks bristles (ROHDE & FAUBEL, 1997a). The bristles of *M. pusillum* and *H. rostratus* clearly are not flagellar derivatives, whereas the much larger bristles of *M. tuba* have been interpreted as modified flagella. It is possible that dense granules in all three species contribute to bristle formation, but that the large size and more complex structure of bristles in *M. tuba* are due to a flagellar component. However, evidence for this assumption is lacking. The possibility must also be considered that the very small bristles of *M. pusillum* and *H. rostratus* are not homologous with the much larger and more complex ones of *M. tuba*. Also, there is a certain similarity between the bristles of *M. pusillum* and the crested bodies of cestodes (BÀ & MARCHAND, 1995), but homology is unlikely in view of the different shape (spiralling around the anterior end of sperm at least in some cestodes). The function of the bristles is unknown. That the curved dense structures in *H. rostratus* may act as « bristles » is indicated by their taper-ing tips close to the surface. Conceivably, the soft surface membrane, when pushed against a hard or elastic surface, permits the tips to find a hold and act as « bristles ».

The observation that many granules (from very small to medium large) in sperm of *H. rostratus* are lined by a distinct membrane, whereas many others of the same size are not, may indicate that loss of a membrane is a fixation artefact. The frequency distribution of diameters of sections through the granules suggests that there are a size class of small granules (average diameter approximately 0.2 μm, slightly greater than the peak of mea-

surements of sections because most sections will not be exactly through the middle of the granules) and many larger granules which probably belong to a size class of their own. The observation that some curved structures occur among the granules in the interior of sperm from the testis, resembling the subsurface "bristles" in mature sperm, suggests that the « bristles » are formed in the interior and migrate to the surface during maturation of the sperm.

ACKNOWLEDGMENTS

REFERENCES

BÂ, C.T. & B. MARCHAND (1995) – Spermiogenesis, spermatozoa and phyletic affinities in the Cestoda. *In*: B.G.M. JAMIESON, J. AUSIO and J.-L. JUSTINE (eds.), Advances in Spermatozoal Phylogeny and Taxonomy, *Mémoires du Muséum National d'Histoire Naturelle*, Paris, 87-95.

BEDINI, C., & F. PAPI (1970) – Peculiar patterns of microtubular organization in spermatozoa of lower Turbellaria. *In*: B. BACCETTI (ed.), Comparative Spermatology, *Accad. Naz. dei Lincei*, Rome, 363-366.

CANNON, L.R.G. (1986) – Turbellaria of the World. A Guide to Families and Genera, Queensland Museum, Brisbane, 136 pp.

DOE, D.A. (1982) – Ultrastructure of the copulatory organs of *Haplopharynx quadristimulus* and its phylogenetic significance. (Platyhelminthes, Haplopharyngida), *Zoomorphology*, **106**: 163-173.

EHLERS, U. (1985) – Das Phylogenetische System der Plathelminthes, Gustav Fischer, Stuttgart, New York, 317 pp.

NEWTON, W.D. (1980) – Ultrastructural analysis of the motile apparatus of the aflagellate spermatozoon of *Macrostomum tubum*, *J. Ultrastruct. Res.*, **73**: 318-330.

ROHDE, K. & A. FAUBEL (1997a) – Spermatogenesis of *Paromalostomum fusculum* (Platyhelminthes, Macrostomida, Dolichomacrostomidae). *Invertebr. Reprod. and Dev.*, **32**: 75-84.

ROHDE, K. & A. FAUBEL (1997b) – Spermatogenesis of *Macrostomum pusillum* (Platyhelminthes, Macrostomatidae), *Invertebr. Reprod. and Dev.*, **32**: 209-215.

ROHDE, K. & N. WATSON (1991) – Ultrastructure of spermatogenesis and sperm of *Macrostomum tuba*, *J. Submicrosc. Cytol. Pathol.*, **23**: 23-32.

WATSON, N.A. & K. ROHDE (1995) – Sperm and spermiogenesis of the « Turbellaria » and implications for the phylogeny of the phylum Platyhelminthes. *In*: B.G.M. JAMIESON, J. AUSIO and J.-L. JUSTINE (eds.), Advances in Spermatozoal Phylogeny and Taxonomy, *Mémoires du Muséum National d'Histoire Naturelle*, Paris, 37-54.

SPERM MORPHOLOGY OF THE DIPODID RODENTS (JERBOAS) COMMON IN EGYPT

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Abstract. Sperm morphology of the jerboas *Allactaga* and *Jaculus* species common in Egypt was described by light and transmission electron microscopy. Two morphs of spermatozoa were observed among these dipodids. One type observed in the smaller jérboa, *A. tetradactyla*, has a paddle-shaped long head, long tail, and the connecting piece of tail inserts off-centre at the base of the sperm head. The other form found in both lesser jerboas, *Jaculus jaculus* subspecies and large jerboa, *Jaculus orientalis orientalis*, has a pear-shaped short head, relatively short tail, and the connecting piece of the tail attaches midbasally to the lower concave surface of the sperm head. In both types, the sperm has nearly the same internal organization and the head is symmetrical, bilaterally flattened, lacks any traces of hooks or processes, and is capped by a massive symmetrical apical acrosomal segment. Accordingly, it was concluded that they are part of one evolutionary radiation and the « symmetrical head-short tail » sperm type represents the ancestral type for dipodid rodents. Moreover, it was shown that the dipodids share many of their sperm features with muroids. Hence, it was suggested that the dipodids during their evolution have formed a clade that has been branched off either from Muroidea or Myomorpha. Of course this association needs more further studies which are under consideration.

Key Words : Sperm, morphology, ultrastructure, Dipodidae, rodents, Jerboas, *Allactaga tetradactyla, Jaculus* spec.

INTRODUCTION

Observations on sperm morphology from most species of mammals have indicated that although nearly all eutherian spermatozoa have the same basic design, the morphology of the head of the mature spermatozoon varies markedly between, and occasionally within, the various mammalian orders. It is generally spatulate, paddle-shaped or pear-shaped, but in most murid rodents it is hook-shaped with the anterior region of the nucleus surrounded by an elaborate acrosome and an extension of the subacrosomal cytoskeleton as a perforatorium (RETZIUS, 1909 ; FRIEND, 1936 ; BISHOP & AUSTIN, 1957 ; FAWCETT, 1970 ; FAWCETT et al., 1971 ; BREED & YONG, 1986 ; BEDFORD & HOSKINS, 1990 ; BREED & MUSSER, 1991 ; BREED & APLIN, 1994 ; EDDY & O'BRIEN, 1994 ; BASKEVICH & LAVRENCHENKO, 1995 ; LAVRENCHENKO & BASKEVICH, 1996 ; BREED, 1991, 1995a, b, 1997). The degree of variation in sperm head shape related to functional requirements is

still unknown. However, as appeared from studies on different mammalian groups, the patterns of similarity and difference in sperm head morphology can be indicative of inter-specific or intergeneric relationships (LINZEY & LAYNE, 1974; HARDING et al., 1979, 1982; TEMPLE-SMITH, 1987; HARDING & APLING, 1990; BREED & APLIN, 1994).

Since recent studies on the spermatozoal morphology of murid rodents from certain continents have indicated a considerable range of morphological types in comparison with those occurring elsewhere (BREED & SARÁFIS, 1979; BREED, 1980, 1983, 1984a, b; BASKEVICH & LAVRENCHENKO, 1995; LAVRENCHENKO & BASKEVICH, 1996), it became pos-sible to use the sperm morphology data to evaluate the taxonomic and phylogenetic rela-tionships and to support the conclusions arrived at from karyotypic and electrophoretic studies (BAVERSTOCK et al., 1981; BREED & YONG, 1986; BREED & MUSSER, 1991; BREED & APLIN, 1994; BREED, 1991, 1995a, b, 1997).

The dipodids, jerboas, in Egypt were classified into two genera: Allactaga Cuvier, 1836 and Jaculus Erxleben, 1777 (OSBORN & HELMY, 1980). The Allactaga has one species, A. tetradactyla (small jerboa), while the Jaculus includes two species, J. orien-talis (greater jerboa) and J. jaculus (lesser jerboa). The latter species, according to OSBORN & HELMY (1980), has four subspecies, J. j. jaculus, J. j. flavillus, J. j. schlueteri, and J. j. butleri.

According to ANDERSON (1967), the Dipodoidea likewise Muroidea and Gliroidea were considered as a fairly distinctive group of Myomorpha. However earlier, the Muroidea were considered the nucleus of the Dipodoidea and Gliroidea (SIMPSON, 1945).

Although some ecological, behavioral, anatomical, and physiological information is available on the jerboas, family Dipodidae, (ELLERMAN, 1942; WASSIF, 1960; EL HILALI & VEILLAT, 1975; OSBORN & HELMY, 1980; LAKHDAR-GHAZAL et al., 1992; 1995a, b), there appears to be no further information on the spermatozoa of North-African Dipodidae or those occurring elsewhere. Thus the present study was undertaken to describe the appearance and organization of spermatozoa of the dipodids common in Egypt, and their significance for discrimination between these morphologically similar jerboas.

MATERIAL AND METHODS

Live adult males of three species and four subspecies of jerboas belonging to two genera common in Egypt were collected during the period of sexual activity (May-June) from the following localities:

- *Allactaga tetradactyla* Lichtenstein, 1823 (Four-toed or small jerboa), n = 4: Mersa Matruh.
- *Jaculus jaculus jaculus* Linnaeus, 1758 (lesser jerboa with orangish back and black tail band incomplete on the underside), n = 5: El-Faiyum,
- *Jaculus jaculus flavillus* Setzer, 1955 (lesser jerboa with brownish back, hind foot length less than 63 mm and ear length more than 23 mm), n = 5: Sidi Barrani,
- *Jaculus jaculus schlueteri* Nehring, 1901 (lesser jerboa with brownish back, hind foot length more than 63 mm and ear length more than 23 mm), n = 5: Ismailia,

- *Jaculus jaculus butleri* Thomas, 1922 (lesser jerboa with brownish back and ear length less than 23 mm), n = 5 : Red Sea area,
- *Jaculus orientalis orientalis* Erxleben, 1777 (Greater jerboa), n = 5 : Mersa Matruh.

The animals were killed in the laboratory and the testes and epididymides were dissected out. Small pieces of cauda epididymides were immediately fixed for about 1½ hr in 3% glutaraldehyde made up in 0.1 M phosphate buffer (pH 7.4) for transmission electron microscopy (TEM). After fixation the samples were washed several times in 0.1 M cacodylate buffer (pH 7.4) and fixed in 1% osmium tetroxide for 1-1½ hr, then rinsed again in buffer, dehydrated by passing through a series of ethanols and embedded in epoxy resin. Thick plastic sections (1 μm) were cut, stained with toluidine blue and when appropriate regions were found, ultrathin sections were obtained and stained with lead citrate and uranyl nitrate. The sections were subsequently observed with JEOL JEM-100CX II and photomicrographs were taken.

In addition, sperm samples from all individuals of each species were also prepared for light microscopy (LM) by extruding the contents from caudae epididymides on a watch glass that contained 3% buffered glutaraldehyde. After fixation, drops of the solution with cellular material were placed on microscope slides. After placing a coverslip on each slide, the spermatozoa were observed under phase contrast optics and photographed with x 100 objective.

Measurements of spermatozoa from each species were taken with an ocular micrometer slide at x 100 magnification. The approximate lengths of the head, midpiece and tail as well as breadth of the head and midpiece were measured as described by BREED & APLIN (1994). The combined length of the principal and end pieces of the tail was taken because they are indistinguishable from each other under the light microscope.

RESULTS

The terminology used for describing orientation of the sperm head and morphology of the head and tail follows BREED (1983, 1984a, b) and FLAHERTY & BREED (1983). However, the terminology of the planes of sections through the sperm follows BREED (1995a, b).

Two major sperm types were observed among the jerboas examined depending on the shape of the head and site of the tail insertion into the head. One type (Fig. 1b) has a paddle-shaped head, a broad face (6.0 μm wide) and a connecting piece of tail that inserts off-centre at the basal concave surface of the sperm head. The other type (Figs 2a ; 3a) has a pear-shaped head, a relatively narrow face (4.0 μm wide) and a connecting piece of tail that attaches to the midbasal concave surface of the sperm head. In both forms, the sperm head appears symmetrical, bilaterally flattened, lacks any traces of hooks or processes, and narrows a little toward the connecting piece of the tail. The acrosome is large and massive ; it is symmetrical with an apical segment forming a cap above the bilaterally flattened nucleus and a thin equatorial segment extending along part of the lateral nuclear surfaces, caudal to which is the postacrosomal dense lamina. There is an invagination of the inner acrosomal membrane, into which the subacrosomal cytoskeleton or perforatorium projects, as a midline apical ridge over the convex nuclear surface. The midpiece is readily

distinguishable from the principal piece of the tail. The ultrastructural organization of the sperm tail resembles that of other rodents.

Furthermore, differences in the dimensions of sperm head and tail among the species studied are considerable (Table 1) and are as follows.

TABLE 1

Sperm dimensions of the dipodoid rodents in Egypt.
The value given is the largest measurement for each dimension;
all values are in micrometers (μm)

| Species | Head | | Tail | | |
	Lenth	Breadth	MP	PP+EP	Total
Allactaga tetradactyla	9	6	15(2)	95	110
Jaculus j. jaculus	7	4	8(1)	50	58
Jaculus j. butleri	6	4	7(1)	50	57
Jaculus j. schlueteri	7	4	8(1)	50	58
Jaculus j. flavillus	7	4	8(1)	49	57
Jaculus o. orientalis	8	4	7(1)	46	53

MP= Midpiece length; the value in brackets is the midpiece breadth. PP+EP= Combined length of principal and end pieces.

Allactaga tetradactyla. In *A. tetradactyla*, the sperm is considerably different from those of *Jaculus* species. The head has a paddle-shape (Fig. 1b); it is 9.0 μm long and 6.0 μm wide. The tail is relatively long; it is 110 μm long, of which 15 μm is distinguishable as midpiece. The midpiece is inserted off-centre in a concave surface at the base of the head (Fig. 1b, c, e, f). The acrosome forms a prominent girdle around the posterior nuclear region (Fig. 1b, c).

TEM sections through sperm head indicate that the nucleus is bilaterally flattened and has a homogeneous electron-dense material; it is thicker basally and thinner apically. Its anterior two-thirds is capped by the thinner equatorial segment of the acrosome (Fig. 1a, d, e, f). In the anterior region of the sperm head the acrosome forms a thick apical segment with a homogeneous electron-dense matrix over the dorsal convex surface of the nucleus; it presumbly corresponds to the principal segment with the thinner equatorial segment being present posteriorly (Fig. 1a, d, e, f). At the anterior tip of the nucleus there is a very

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Legend to the figures (see page 193)

Fig. 1. – Sperm morphology of *Allactaga tetradactyla*. b. Phase-contrast micrograph. a, c-f. TEM micrographs showing sperm ultrastructure. (a) Scale bar = 0.23 μm, x 10 000; (b) Scale bar = 1.33 μm, x 1 350; (c) Scale bar = 0.08 μm, x 17 300; (d) Scale bar = 0.23 μm, x 10 000: (e) Scale bar = 0.13 μm, x 17 000; (f) Scale bar = 0.98 μm, x 14 000. Abbreviations in this and the following plates are: Ac= acrosome; CP= connecting piece of the tail; ES= equatorial segment of the acrosome; MP= midpiece of the tail; N= nucleus; P= perforatorium; Pl= plasmalemma; PADL= postacrosomal dense lamina; T= tail of the sperm.
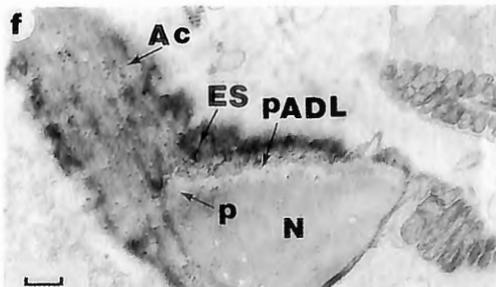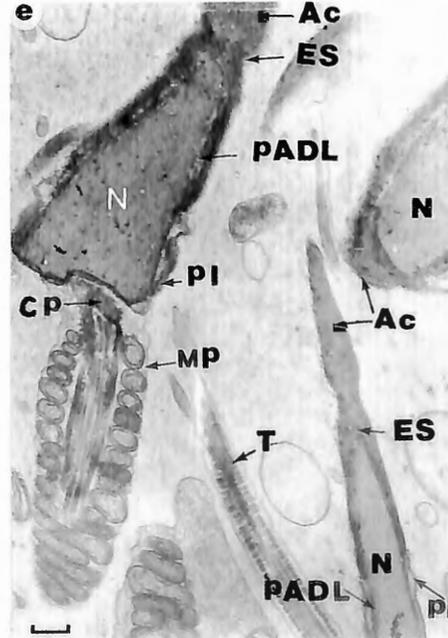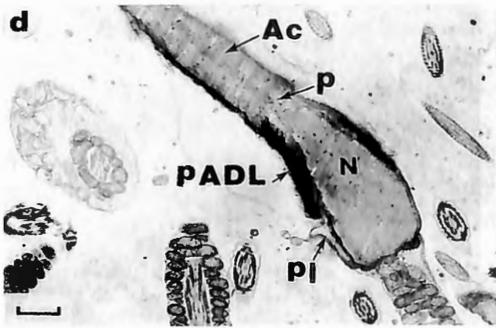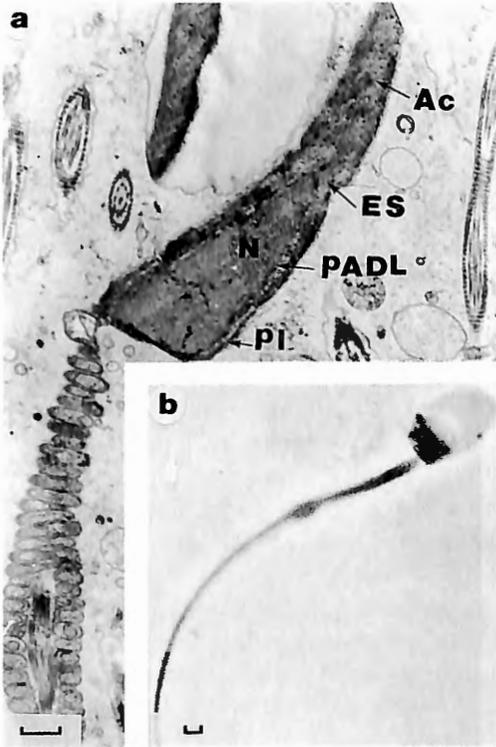
small accumulation of the subacrosomal material which passes a little down between the outer nuclear envelope and inner acrosomal membrane (Plate 1d). In the posterior region of the sperm head there is a relatively short postacrosomal dense lamina which is tightly bound to overlying plasmalemma (Fig. 1a, d, e, f).

Jaculus species. LM observations of spermatozoa from the two species of *Jaculus, J. jaculus* and *J. orientalis*, indicate marked similarity in form. The head is pear-shaped, i.e., it narrows slightly posteriorly, and the tail attaches to the lower midbasal concave surface of the nucleus (Figs 2a; 3a). The acrosome, in common with that of *A. tetradactyla*, is symmerical; it has a large apical acrosomal segment that forms a cap over much of the head surface. The tail is relatively short. However, variations in the head and tail dimensions occur between the two species (Table 1).

In *J. jaculus* subspecies, the sperm head ranges from 6.0 to 7.0 µm in length and is about 4.0 µm wide. The midpiece is readily distinguishable from the principal piece; it is from 8.0 to 9.0 µm long and 1.0 µm wide. The principal and end pieces combined are from 49.0 to 50.0 µm long.

In *J. orientalis*, however, the head is usually longer; being 8.0 µm in length, but in common with *J. jaculus*, it is 4.0 µm in width and has no hooks or processes. The tail measures 53 µm, of which the midpiece is 7.0 µm.

TEM sections through the spermatozoa of *J. jaculus* subspecies as well as *J. orientalis* indicate that the bilaterally flattened head has a homogenous electron-dense nucleus that tapers apically; only one-third of its covex margin is capped by the equatorial segment of the acrosome (Figs 2b, c, d; 3b, c, d). There is a large apical acrosomal segment with a narrower principal segment over the anterior part of the nucleus, posterior to which the equatorial segment passes down over the convex nuclear margin with the postacrosomal dense lamina passing around the posterior caudal margin (Figs 2b, d, e; 3b, c, d). The postacrosomal dense lamina is relatively long in comparison with that present in *A. tetradactyla*, and comes also in direct contact with the plasmalemma. The plasmalemma as in *A. tetradactyla* is wavy over the acrosomal region, but tightly bound to the underlying structures in the postacrosomal region (Figs 2b, c, d; 3b). The acrosomal matrix has a homogeneous electron-dense structure. A moderate subacrosomal space with a less electron-dense material occurs between the inner acrosomal membrane and outer nuclear envelope (Figs 2b, c, d, e; 3b, c, d, e).

Legend to the figures (see pages 195-196)

Fig. 2. – Sperm morphology of *Jaculus jaculus jaculus* (as example of *Jaculus jaculus* subspecies). a. Phase-contrast micrograph. b-e. TEM sections through the sperm showing sperm ultrastructure. (a) Scale bar = 1.33 µm, x 1 350; (b) Scale bar = 0.15 µm, x 20 000; (c) Scale bar = 0.15 µm, x 20 000; (d) Scale bar = 0.15 µm, x 20 000; (e) Scale bar = 0.33 µm, x 10 000.

Fig. 3. – Sperm morphology of Jaculus orientalis orientalis. a. Phase-contrast micrograph. b-e. TEM micrographs showing sperm ultrastructure. (a) Scale bar=1.33 µm, x 1 350; (b) Scale bar = 0.20 µm, x 20 300; (c) Scale bar = 0.13 µm, x 27 000; (d) Scale bar = 0.15 µm, x 20 000; (e) Scale bar = 0.06 µm, x 40 000.
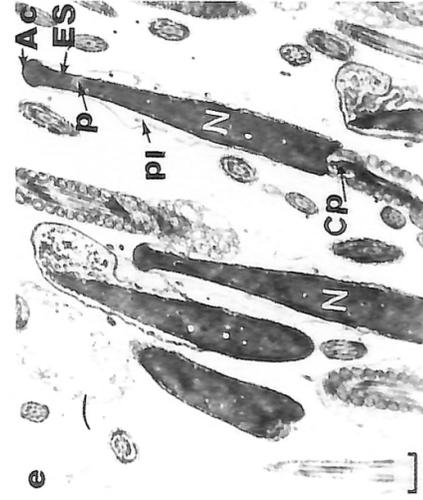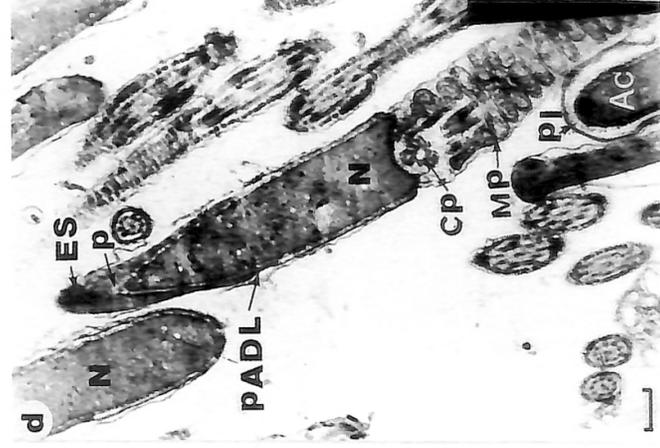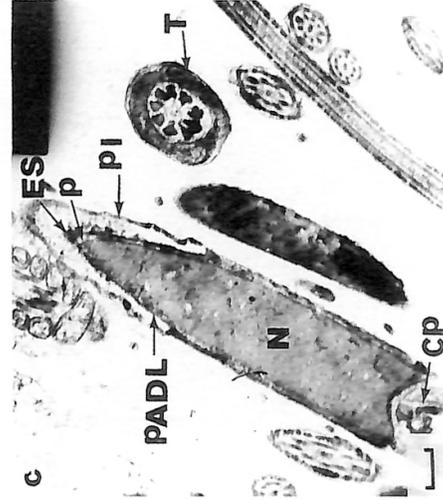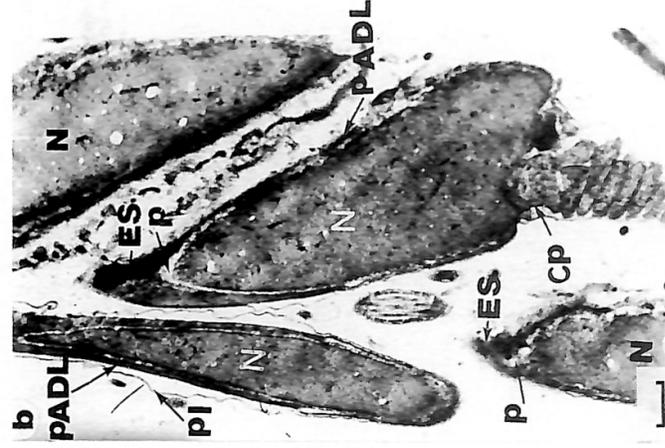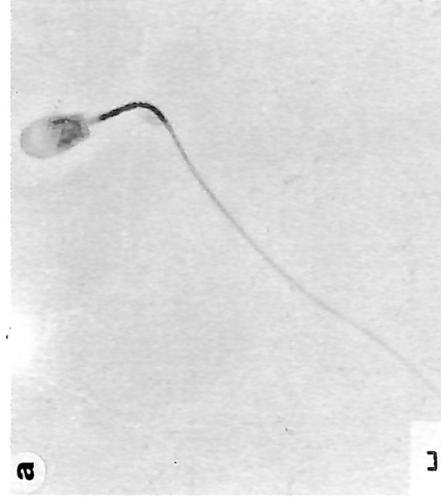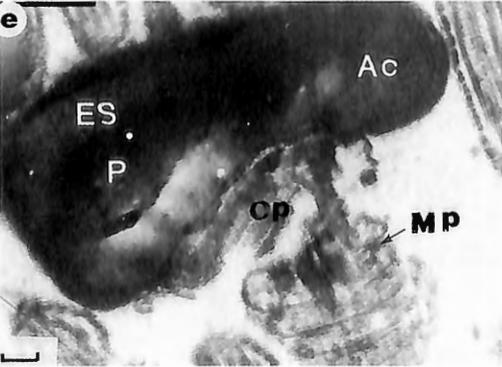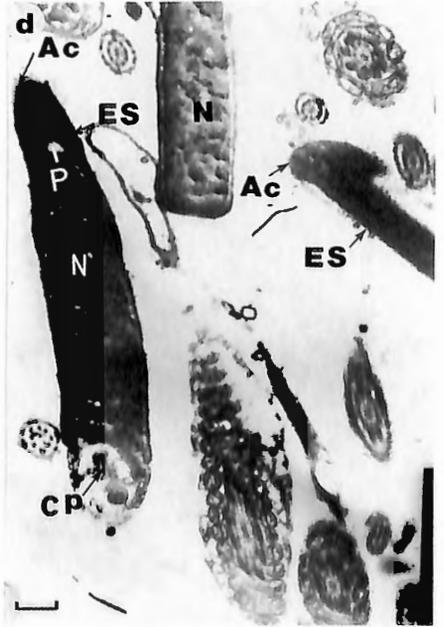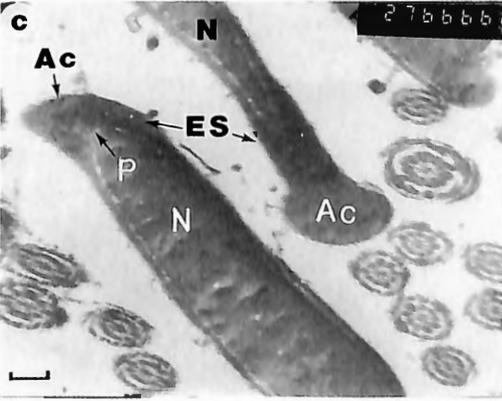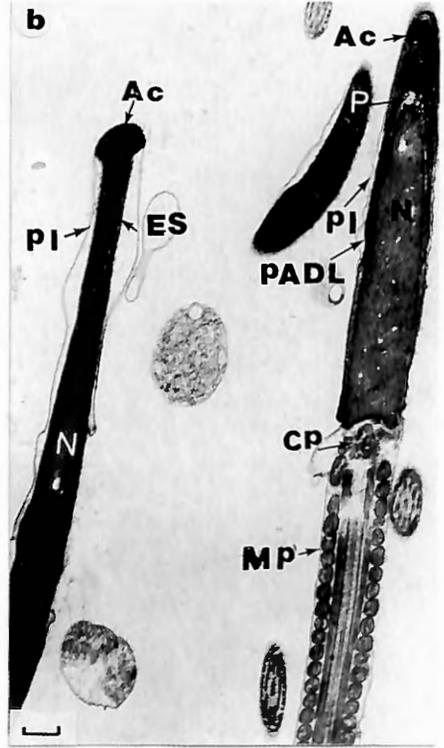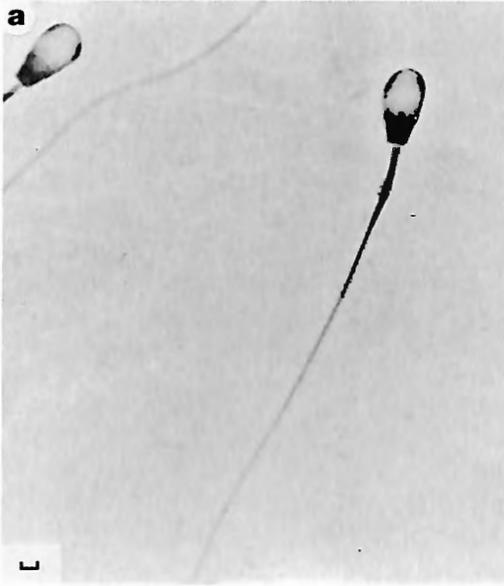
DISCUSSION

Two spermatozoal types were observed among the dipodids examined. The first is found in *A. tetradactyla* and typified by a paddle-shaped long head, long tail, and the connecting piece of tail inserts eccentric or off-centre at the lower concave surface of the head. The second type is found in both *J. jaculus* subspecies and *J. orientalis* and characterized by a pear-shaped short head, and short tail that attaches at the lower midbasal region of the head. These two morphs may be alternations of a single morphological type; the plesiomorphic type (BREED & MUSSER, 1991). In both cases the sperm head is bilaterally flattened and symmetrical with a basally attached, and relatively short, sperm tail.

ROLDAN *et al.* (1992) concluded from the wide distribution among myomorph and perhaps caviomorph rodents of the plesiomorphic sperm head that lacks an apical hook and is associated with a shorter tail, that it represents the ancestral condition for Rodentia as a whole and is basal to each of the major radiations within Myomorpha. Moreover, they further postulated that the elongate falciform head form was apomorphic and had evolved on a number of different occasions within the Muroidea.

Generally, the considerable heterogeneity in sperm head morphology does not show any obvious adaptive significance, *i. e.*, not to be related to ecology or life history and may have evolved by random genetic drift (AUSTIN, 1976; BREED & YONG, 1986). Accordingly, sperm morphology may be a useful independent character for gleaning information about the genealogical relationships when considered in relation to other morphological, biochemical, and karyotypic data (BREED & YONG, 1986). The site of attachment of the sperm tail to the head and the occurrence of the subacrosomal cytoskeleton or perforatorium beneath the acrosome vary markedly between rodents (BREED & YONG, 1986; BREED & MUSSER, 1991; BREED, 1991, 1995a, b). As described by BREED (1995b), the falciform sperm nucleus is always associated with a highly developed cytoskeleton, presence of a perforatorium, asymmetrical acrosome, and a relatively long tail that usually attaches to the lower concave surface of the sperm head. Conversely, a more symmetrical nucleus is accompanied by a simpler structural organization of the cytoskeleton, sometimes a relatively short tail that attaches basally, and a more symmetrical acrosome. An interpretation of this association of morphological traits is still obscure, although BREED (1995b) suggested that the occurrence of highly developed and elaborate cytoskeletal structures and long sperm tails of the falciform-shaped sperms may be adaptations for chromatin protection needed during the physical thrust of the sperm through the zona pellucida. On the other hand, spermatozoa with a poorly developed cytoskeleton, partly condensed chromatin, and large acrosome, as described by BREED (1995b) and observed in the present study, may occur where a large hole is digested in the zona as a result of the release of acrosomal contents without need for any physical thrust.

In conclusion, the present data on sperm morphology demonstrate that the two species *J. jaculus* and *J. orientalis* are more similar to each other than either of them to *A. tetradactyla*. However, the similarity of the internal structural organization of sperm in these three dipodids suggest very strongly that they are part of one evolutionary radiation and this « symmetrical head-short tail » sperm type represents the ancestral condition for dipodid rodents. Moreover, the dipodids in Egypt have many of muroid sperm features.

Therefore, they are not so apart from muroid rodents and may form a clade that is either an offshoot of Muroidea (SIMPSON, 1945) or may have branched off from the Myomorpha (ANDERSON, 1967). Furthermore, sperm morphology provides good taxonomic evidence for discrimination between these externally similar jerboas.

ACKNOWLEDGMENTS

REFERENCES

ANDERSON, S. (1967) – Introduction to the rodents. In : *Recent mammals of the world: a synopsis of families*. ANDERSON, S. & J.K. JONES, Jr. (Eds.). New York : Ronald Press Co., 206-209.

AUSTIN, C.R. (1976) – Specialization of gametes. In : *The evolution of reproduction*. Cambridge Univ. Press, **6** : 149-182.

BASKEVICH, M.I. & L.A. LAVRENCHENKO (1995) – On the morphology of spermatozoa in some African murines (Rodentia, Muridae) : the taxonomic and phylogenetic aspects. *J. Zool. Syst. Evol. Research*, **33** : 9-16.

BAVERSTOCK, P.R., C.H.S. WATTS, M. ADAMS & S.R. COLE (1981) – Relationships among Australian rodents (Muridae). *Aust. J. Zool.*, **29** : 289-303.

BEDFORD, J.M. & D.D. HOSKINS (1990) – The spermatozoon : morphology, physiology and biochemistry. In : MARSHALL's *physiology of reproduction. The male reproductive system*. LAMMING, G.E. (Ed.). London : Churchill Livingstone, **2** : 379-568.

BISHOP, M.W.H. & C.R. AUSTIN (1957) – Mammalian spermatozoa. *Endeavour*, **16** : 137-150.

BREED, W.G. (1980) – Further observations on spermatozoal morphology and male reproductive tract anatomy of *Pseudomys* and *Notomys* (Mammalia : Rodentia). *Trans. R. Soc. S. Aust.*, **104** : 51-5.

BREED, W.G. (1983) – Variation in sperm morphology in the Australian rodent genus *Pseudomys* (Muridae). *Cell Tissue Res.*, **229** : 611-625.

BREED, W.G. (1984a) – Sperm morphology of New Guinea rodents-phylogenetic implications. *Aust. Mammal Soc. Bull.*, **8** (2) : 101 pp.

BREED, W.G. (1984b) – Sperm head structure in the *Hydromyinae* (Rodentia : Muridae) : a further evolutionary development of the subacrosomal space in mammals. *Gamete Res.*, **10** : 31-44.

BREED, W.G. (1991) – Variation in sperm head morphology of the Murinae (Family Muridae) : some taxonomic implications. In : *Comparative spermatology: 20 years after*. BACCETTI, B. (Ed.). New York : Raven Press : 937-939.

BREED, W.G. (1993) – Novel organization of the spermatozoon in two species of murid rodents from southern Asia. *J. Reprod. Fert.*, **99** : 149-158.

BREED, W.G. (1995a) – Variation in sperm head morphology of Muroid rodents of Africa : phylogenetic Implications. In : *Advances in spermatozoal phylogeny and Taxonomy*. JAMIESON, B.G.M., J. AUSIO & J.-L. JUSTINE (Eds.). Paris : Mém. Mus. natn. Hist. nat., **166** : 409-420.

BREED, W.G. (1995b) – Spermatozoa of murid rodents from Africa : morphological diversity and evolutionary trends. *J. Zool. Lond.*, **237** : 625-651.

BREED, W.G. (1997) – Evolution of the spermatozoon in Australian rodents. *Aust. J. Zool.*, **45**: 459-478.

BREED, W.G. & K. APLIN (1994) – Sperm morphology of murid rodents from New Guinea and the Solomon Islands: phylogenetic implications. *Aust. J. Zool.*, **43**: 17-30.

BREED, W.G. & G.G. MUSSER (1991) – Sulawesi and Philippine rodents (Muridae): a survey of spermatozoal morphology and its significance for phylogenetic inference. *Am. Mus. Novit.*, **3003**: 1-15.

BREED, W.G. & V. SARAFIS (1979) – On the phylogenetic significance of spermatozoal morphology and male reproductive tract anatomy in Australian rodents. *Trans. R. Soc. S. Aust.*, **103**: 127-139.

BREED, W.G. & H.S. YONG (1986) – Sperm morphology of murid rodents from Malaysia and its possible phylogenetic significance. *Am. Mus. Novit.*, **2856**: 1-12.

EDDY, E.M. & D.A. O'BRIEN (1994) – The spermatozoon. In: *The physiology of reproduction*, 2nd edn. KNOBIL, E. & J.D. NEILL (Eds). New York: Raven Press: 29-77.

EL HILALI, M. & J.P. VEILLAT (1975) – Jaculus orientalis: A true hibernator. Mammal., **39**: 402-404.

ELLERMAN, J.R. (1942) – Families and genera of living rodents. *Brit. Mus. Nat. Hist.* London, **2**:1-690.

FAWCETT, D.W. (1970) – A comparative view of sperm ultrastructure. *Biol. reprod.*, (Suppl.) **2**: 90-127.

FAWCETT, D.W., W.A. ANDERSON & P.M. PHILLIPS (1971) – Morphogenetic factors influencing the shape of the sperm head. *Devl. Biol.*, 28: 220-251.

FlAHERTY, S.P. & W.G. BREED (1983) – The sperm head of the plains mouse *Pseudomys australis*: ultrastructure and effect of chemical treatments. *Gamete Res.*, **8**: 231-244.

FRIEND, G.F. (1936) – The sperms of British Muridae. *Q. J. microsc. Sci.*, **78**: 419-443.

HARDING, H.R. & K.P. APLIN (1990) – Phylogenetic affinities of the koala (Phascolarctidae; Marsupialia): a reassessment of the spermatological evidence. In: *Biology of the koala*. LEE A.K., HANDASYDE, K.A. & G.D. SANSON (Eds.). Sydney: Surrey Beatty: 1-31.

HARDING, H.R., F.N. CARRICK & D.C. SHOREY (1979) – Special features of sperm structure and function in marsupials. In: *The spermatozoon*. FAWCETT, D.W. & J.M. BEDFORD (Eds.). Urban and Schwarzenberg: Baltimore: 289-303.

HARDING, H.R., P.A. WOOLLEY, D.C. SOREY & F.N. CARRICK (1982) – Sperm ultrastructure, spermiogenesis, phylogenetic implications. In: *Carnivorous marsupials*. ARCHER, M. (Ed.). Sydney: R. Zool. Soc. New South Wales, **2**: 659-673.

LAKHDAR-GHAZAL, N., M. DUBOIS-DAUPHIN, M.L.H.J. HERMES, R.M. BUIJS, W.A. BENGELLOUN & P. PÉVET (1995a) – Vasopressin in the brain of a desert hibernator, the jerboa (*Jaculus orientalis*): Presence of sexual dimorphism and seasonal variation. *J. Comp. Neurol.*, **358**: 499-517.

LAKHDAR-GHAZAL, N., A. KALSBEEK & P. PÉVET (1992) – Sexual differences and seasonal variations in vasoactive intestinal peptide immunoreactivity in the suprachiasmatic nucleus of jerboa (*Jaculus orientalis*). *Neurosci. Lett.*, **144**: 29-33.

LAKHDAR-GHAZAL, N., R. OUKOUCHOUD & P. PÉVET (1995b) – Seasonal variation in NPY immunoreactivity in the suprachiasmatic nucleus of the jerboa (*jaculus orientalis*), a desert hibernator. *Neurosci. Lett.*, **193**: 49-52.

LAVRENCHENKO, L.A. & M.I. BASKEVICH (1996) – Variation of generative system structure in some species of the genus *Mastomys* Thomas, 1915 (Rodentia, Muridae). Mammalia, **60**: 277-288.

LINZEY, A.V. & J.N. LAYNE (1974) – Comparative morphology of spermatozoa of the rodent genus *Peromyscus* (Muridae). *Am. Mus. Nov.*, **2532**: 1-20.

OSBORN, D.J. & I. HELMY (1980) – The contemporary land mammals of Egypt (including Sinai). – *Fieldiana Zool.* No. 5 : 579 pp.

RETZIUS, G. (1909) – Spermatozoa of mammals. Biol. Unters., (NF) **14** : 163-178.

ROLDAN, E.R.S., M. GOMENDIO & A.D., VITULLO (1992) – The evolution of eutherian spermatozoa and underlying selective forces : female selection and sperm competition. *Biol. Rev.*, **67** : 551-593.

SIMPSON, G.G. (1945) – The principles of classification and a classification of mammals. – *Bull. Am. Mus. Nat. His.* New York, **85** : 1-350.

TEMPLE-SMITH, P.D. (1987) – Sperm structure and marsupial phylogeny. In : *Possums and Opossums : studies in evolution.* ARCHER, M. (Ed.). Sydney : R. Zool. Soc. New South Wales, **1** : 172-194.

WASSIF, K. (1960) – Studies on the osteology of Egyptian Jerboas. – *Bull. Zool. Soc. Egypt*, **15** (29) : 71- 92.

LARVAL HEMOLYMPH FEEDING AND OOPHAGY:
BEHAVIOR OF QUEEN AND WORKERS
IN THE PRIMITIVE PONERINE ANT
PRIONOPELTA KRAEPELINI
(HYMENOPTERA, FORMICIDAE)

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Abstract. The behavior of the queen and workers in the amblyoponine ant *Prionopelta kraepelini* was observed and compared with that of the queen and workers of *P. amabilis*. The queen fed mainly on larval hemolymph by pinching the larval body. Workers often laid trophic eggs, most of which were given to larvae. The foraging and recruitment behavior of workers were similar to *P. amabilis*.

Key words: ants, Ponerinae, *Prionopelta*, oophagy, recruitment, larval hemolymph feeding

INTRODUCTION

The ant tribe Amblyoponini represents an important group for understanding the diversity of ant behavior, since they are morphologically the most primitive of the Ponerinae (HÖLLDOBLER & WILSON, 1990). It remains controversial, however, whether they are a primitive or derived group (HASHIMOTO, 1996). Their bionomic information is still largely lacking, because most species are distributed in the tropics and are very rare (BROWN, 1960). *Prionopelta* Mayr, 1866 is a genus of small amblyoponine ants, the bionomics and foraging behavior of which are known only from the neotropical *P. amabilis* Borgmeier, 1949. This species has very special behavioral and morphological characteristics: queens feed only on trophic eggs laid by workers, the pupal chamber shows « wall papering » of the surface with cocoon fragments, and workers show recruitment of nestmates for prey retrieval by using a trail pheromone which originates from special basitarsal glands in the hindlegs (HÖLLDOBLER & WILSON, 1986; HÖLLDOBLER *et al.*, 1992). However, it is still unknown whether these characteristics are typical for this species only, or whether they also apply to other species in this genus.

We collected a colony of the very rare *Prionopelta kraepelini* Forel, 1905 in the type locality of this species, the Bogor Botanic Gardens (West Java, Indonesia). In the laboratory, we observed the behavior of queen and workers, and examined the morphology of

the leg glands of workers. In this paper, we describe our findings on *Prionopelta krae-pelini*, and compare this species with the neotropical *P. amabilis*.

MATERIAL AND METHODS

The colony was collected in the Bogor Botanic Garden, Bogor (6°35'S, 106°47'E), West Java, Indonesia in December 1995. Judging from the revision of BROWN (1960), the specimens were identified as *Prionopelta kraepelini* (TERAYAMA, pers. comm.), which was originally described from the same locality. The voucher specimens were deposited in the Bogor Zoological Museum.

The colony was kept in an artificial nest box of 10 x 6.3 x 2.5 cm. The bottom of the nest was covered with plaster of Paris mixed with activated carbon powder. A brood chamber was excavated in the centre of the plaster floor, the top of the chamber was covered with clear glass. Small crickets were fed as prey. The nest was kept at 24 to 28° C. Water was often applied to the plaster floor to maintain high humidity. Because of the small body size of this species, all observations were carried out under a binocular dissecting microscope. During the observations, the number of workers ranged between 80 and 100, while the colony also contained a few males and all stages of brood. Queen behavior was observed for a total of 21h20 min during one month. Each observation period lasted 20 to 40 min. All behavioral acts performed by the queen were observed during this period. The foraging behavior of workers was observed four times. For each observation, we recorded the number of workers in the foraging arena every 5 min during 1 hour. Then, a small cricket was offered in the for-aging arena. Counting the number of workers in the foraging arena and around the prey was continued for 1 hour. After these observations, the queen and several workers were dissected to check their reproductive condition.

Hindlegs of workers were fixed in 2% glutaraldehyde, buffered at pH 7.3 with 0.05 M Na-cacodylate and 0.15 M saccharose. After postfixation in 2% osmium tetroxide, tissues were dehydrated in acetone and embedded in Araldite. Thin sections were double stained with a LKB Ultrostainer, and examined with a Zeiss EM 900 transmission electron micro-scope. Worker hindlegs for scanning microscopy were coated with gold and viewed with a Philips SEM 515 microscope.

RESULTS

Nest and colony composition

The colony of *P. kraepelini* was collected from a moist, dead stump in the Bogor Botanic Gardens. The stump was inhabited by a colony of *Leptogenys diminuta* (F. Smith, 1857) but also contained two nest chambers of *P. kraepelini*, from which we collected all individuals. Chamber A contained a dealate queen and ca. 30 workers with larvae. The queen was immediately dissected: she was virgin without mature oocytes and yellow bodies. Chamber B contained a dealate, mated queen and ca. 60 workers with eggs, larvae and pupae. The wall of the chamber was covered with cocoon fragments. It was not sure

whether the two chambers may have been interconnected by a tunnel. Behavioral observations were carried out for the queen and workers in chamber B. Apart from this colony, we found only one other colony fragment of this species during our annual investigation of the ant fauna of the Bogor Botanic Gardens since 1990. This other colony fragment was equally found in a dead and moist stump. Behavioral observations were carried out for the queen and workers in chamber B.

Queen behavior

The behavioral repertory of the queen is given in Table 1. In all, 14 behavioral acts were recognized. Unlike queens of *P. amabilis* that feed only on trophic eggs laid by workers (HÖLLDOBLER & WILSON, 1986), the queen of *P. kraepelini* fed on prey insects, larval hemolymph, and trophic eggs laid by workers. Feeding on prey brought into the nest was observed only twice, and represented 3.0% of the feeding activity (total time budget to feeding activity was 88 min 36 s), whereas 14.8% occurred as oophagy and 87% through larval hemolymph feeding (LHF). The manner of LHF is very similar to that of *Amblyopone silvestrii* (Wheeler, 1928) (MASUKO, 1986). However, the queen of *P. kraepelini* pinched over the whole body of the larva unlike *A. silvestrii,* in which the queens mainly pinched on the 4th and 5th larval segments (MASUKO, 1986). As in *A. silvestrii,* larvae subjected to LHF did not die from their wounds.

TABLE 1

Behavioral catalogue of the P. kraepelini *queen during 21h 20 min of observations*

Behavioral acts	Frequency	%
self grooming	117	22.8
allogrooming		
received from workers	115	22.4
received from males	1	0.2
toward workers	11	2.1
antennation		
toward workers	132	25.7
toward males	1	0.2
from workers	87	16.9
from males	4	0.8
biting workers	20	3.9
licking larvae	15	2.9
larval hemolymph feeding	25	4.9
licking prey	2	0.4
oophagy	2	0.4
oviposition	2	0.4
Total	534	100

Oophagy was observed 11 times : two eggs were eaten by the queen, one by a male, and eight by larvae, indicating that trophic eggs are mainly used for larval nutrition. We confirmed by direct observation that four of the eggs were laid by workers. Their eggs were evidently smaller than those laid by the queen. After ovipositon of the egg, a worker picked it up from the tip of her abdomen with the mandibles, and walked in the nest chamber for 1 to 3 min before presenting it to a nestmate. When giving the egg, the worker held it with her mandibles and the egg was put on the mouthparts of the nestmate until the end of feeding.

Queens had three ovarioles per ovary. Oviposition by the queen was observed twice. The queen never picked up eggs from the abdominal tip with her mandibles. After oviposition, eggs were laid on the nest floor, and subsequently a worker grasped the egg and brought it to the egg pile. Egg care by the queen was never observed, but was only performed by workers.

The queen frequently received grooming from workers. She sometimes groomed workers, however, the frequency of this behavior was low. Antennation towards and received from workers was often observed. After antennation to workers, the queen sometimes bit the worker's head or mandibles. In *P. amabilis*, the queen was surrounded most of the time by a retinue of ca. 10 workers and was frequently groomed by them (HÖLLDOBLER & WILSON, 1986). In *P. kraepelini*, such worker retinue was not observed.

Worker behavior

Workers foraged individually. Before prey was given, between 7 and 16 workers were found in the foraging arena (Fig. 1). When a worker found a prey insect in the foraging
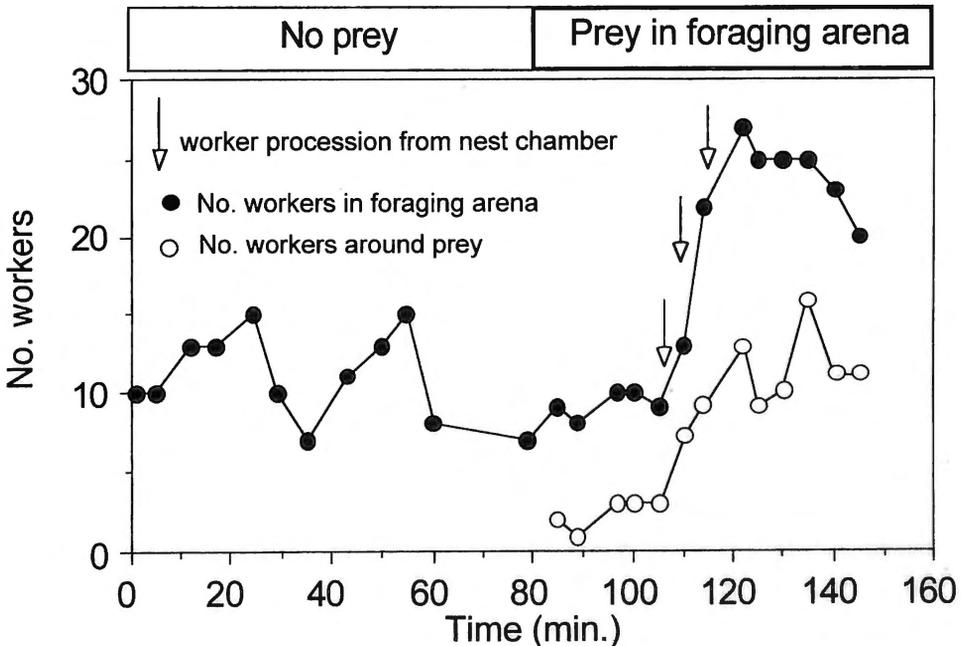

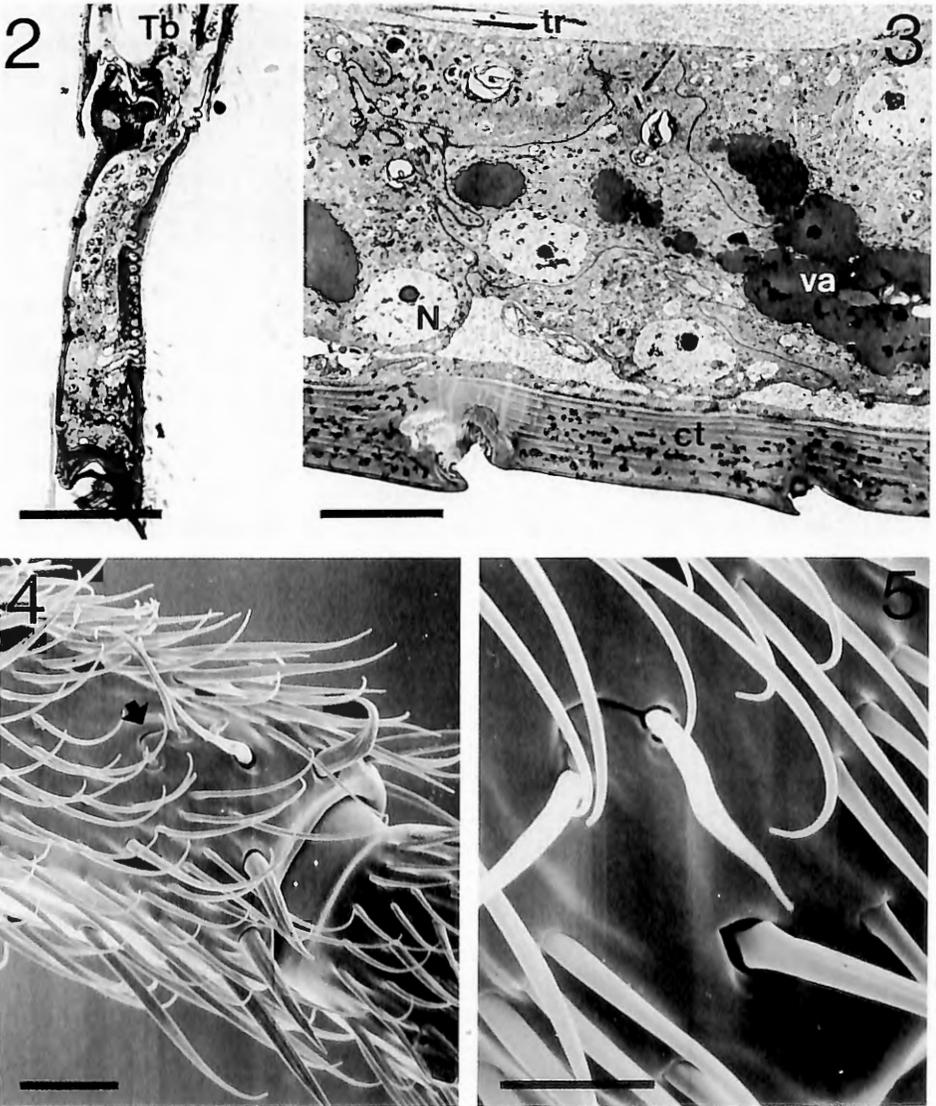Fig. 1. – Number of foraging workers of *P. kraepelini* before and after prey was offered.

Fig. 2. – Semithin section through foreleg basitarsus, showing position of antenna cleaner gland. Cuticular pores correspond with nerve endings associated with gland. Tb = tibia (scale bar 50 μm).

Fig. 3. – Electron micrograph of secretory cells in hindleg basitarsus. ct = cuticle, N = nuclei, tr = tracheoles, va = vacuolar areas (scale bar 5 μm).

Fig. 4. – Scanning electron micrograph of ventrodistal portion of hindleg basitarsus indicating position of ventrally occurring slit (arrow) (scale bar 10 μm).

Fig. 5. – Detail showing semi-circular slit in between two associated hairs (scale bar 5 μm).

arena, she opened her mandibles, and slowly approached the prey. Then, she grasped its appendages and stung. During this interaction with prey, a few workers usually joined in the prey attack. After that, one or a few workers went to the nest chamber and recruited nestmates to the prey site. The number of workers recruited by one recruitment episode was small, up to seven workers made a single procession, and followed the route of scout workers. Such recruitment was repeated one to four times for retrieving one prey. Beside the procession, single workers also went out and followed the trail. Worker behavior during this process was very similar to *P. amabilis*: recruiting workers dragged their hind legs, and showed vertical body shaking when they entered the nest (see HÖLLDOBLER *et al.*, 1992, Fig. 1). Like *P. amabilis*, foraging workers after entering the nest often displayed self-grooming, especially by stroking the hindlegs with the forelegs. Preliminary morphological examination of the limited material available revealed the presence of glandular cells in the basitarsus of both the fore- and hindlegs. In the frontleg basitarsi, densely packed secretory cells occur (Fig. 2), that correspond with the antenna cleaner gland as described by SCHÖNITZER and co-workers (1996). In the hindlegs, polymorphic glandular cells with a rounded nucleus and large vacuolar areas occur (Fig. 3), although we were unable to trace their precise structural contact with the outside. In the scanning microscope, however, we observed on the ventral side the presence of a narrow semi-circular slit occurring between two hairs in the distal portion of the hindleg basitarsus (Figs 4, 5), similar to the situation reported for *P. amabilis* by HÖLLDOBLER *et al.* (1992), who found this to be the site where the basitarsal gland opens. These two hairs are shorter than the surrounding leg hairs, and are situated approx. 4 μm from each other (Fig. 5).

As already mentioned, some workers laid trophic eggs. After the observations, we examined ovarian development in 9 foraging and 10 domestic workers. Judging from their pigmentation, 6 of the latter were apparently young individuals. All workers had one ovariole per ovary. All foraging workers had no developing oocytes while all but one domestic worker had one or two developing oocytes, suggesting that trophic eggs were laid by the domestic workers.

Brood care was similar to the description by HÖLLDOBLER & WILSON (1986). In their artificial nest, workers sometimes put cocoon fragments on the glass ceiling cover, which looks like « wall-papering » behavior. The tips of cocoons of pupae was usually opened: workers may remove the meconium of larvae after they become prepupae. Workers sometimes removed cocoons of young white pupae. Such pupae were laid on the nest floor and emerged later. Adult transport was never observed, although the nest was disturbed by removing the covering glass cover. On such occasions, the queen walked by herself.

DISCUSSION

Comparison of the bionomics between *P. kraepelini* and *P. amabilis* based on the present study and studies by HÖLLDOBLER and co-workers (1986, 1992) remains fragmentary, because for both rare species a very limited number of colonies was available for study. However, the similarity in several aspects of bionomics, e.g. the « wall papering » of the nest chamber, the foraging and recruitment behavior, and the trophic egg production by workers, may indicate that these behaviors are common characteristics of the genus

Prionopelta. Besides in *Prionopelta*, the peculiar «wall papering» with cocoon fragments has only been found in *Harpegnathos saltator* Jerdon, 1851, where this behavior may contribute to stabilizing humidity inside the nest chambers (PEETERS *et al.*, 1994).

Both species also display apparently similar exocrine glands in the basitarsi of both their fore- and hindlegs. The glandular cells in the foreleg basitarsus are thought to be associated with the tibio-tarsal cleaning apparatus (HÖLLDOBLER *et al.*, 1992; SCHÖNITZER *et al.*, 1996). In *P. amabilis*, the hindleg basitarsal gland is reported to produce trail following substances during recruitment to food sources or new nest sites (HÖLLDOBLER *et al.*, 1992). In *P. kraepelini*, a similar function may be likely, as we could clearly demonstrate worker recruitment to prey in this species.

A remarkable difference between both species, however, is found in the queen behavior. HÖLLDOBLER & WILSON (1986) reported that the queen of *Prionopelta amabilis* exclusively fed on trophic eggs, which might be laid by workers. They observed oophagy three times, the three eggs being eaten by the reproductive queen, a virgin queen, and a larva. In *P. kraepelini*, we observed oophagy of 11 trophic eggs, only two of these were eaten by the queen while most eggs were fed to larvae, suggesting that the primary role of the trophic eggs in this species is food for larvae. Time budget data suggest that larval hemolymph is the most important food source for the queen of *P. kraepelini* as is also the case for *Amblyopone silvestrii* (MASUKO, 1986). Since the observation period was rather short for *P. amabilis* (5 hours, HÖLLDOBLER & WILSON, 1986), it is difficult to conclude whether this is a significant difference between the two *Prionopelta* species. In *P. amabilis*, presentation of trophic eggs by workers was very often observed, and up to 5 workers simultaneously present their eggs to the queen (HÖLLDOBLER & WILSON, 1986), while such high frequency of egg presenting was not found in *P. kraepelini*. The reason for this difference may be related to the number of workers: colony size is much smaller in *P. kraepelini* (ca. 100 workers) than in *P. amabilis* (more than 500 workers).

Larval hemolymph feeding (LHF) has been reported for two genera of ponerine ants (*Amblyopone silvestrii* and three species of *Proceratium* Roger, 1863) and the leptanilline ant *Leptanilla japonica* Baroni Urbani, 1977 (MASUKO, 1986, 1989). These species are all specialized predators in which no regurgitation nor trophic eggs are known. *A. silvestrii* and *L. japonica* hunt for geophilomorph centipedes, while *Proceratium* lives from arthropod eggs. Such prey items are often difficult to obtain (due to the large size of centipedes, and parental care of arthropod eggs), which may have an important bearing on the occurrence of LHF as an aberrant feeding mode (MASUKO, 1986, 1989). In contrast to these species, the queen of *Prionopelta kraepelini* predominantly feeds on larval hemolymph even though the workers can lay trophic eggs. Food specialization in *Prionopelta* is apparently less developed than in *A. silvestrii*, *L. japonica* and *Proceratium*. Under laboratory conditions, *P. kraepelini* workers attacked and brought termites, crickets and mealworm larvae to the nest chamber. For *P. amabilis*, a preferential diet of campodeid diplurans has been reported, although they also accept other small arthropods (HÖLLDOBLER & WILSON, 1986). The possibility has been mentioned that LHF may also have a function in control of caste differentiation, in which the queen controls the development of female larvae into workers by LHF (MASUKO, 1986). If this would also be the case for *P. kraepelini*, the occurrence of both LHF and trophic egg production would imply queen-worker conflict

for the production of workers and gynes : queens prefer more to invest in colony mainte-
nance (production of workers) than in production of sexuals, while workers show no pref-
erence for these fractions of investment (BOURKE & FRANKS, 1996).

HÖLLDOBLER & WILSON (1986) listed the similarities and differences between
Amblyopone and *Prionopelta,* based on observations of *A. pallipes* (Haldeman, 1844)
(TRANIELLO, 1982) and *P. amabilis.* Subsequently, detailed studies of the behavior of the two
amblyoponine genera have been published for *A. silvestrii* and *A. reclinata* (MASUKO, 1986,
1996 ; ITO, 1993a,b) and for *P. amabilis* (HÖLLDOBLER *et al.,* 1992). HÖLLDOBLER & WILSON
(1986) stressed that *Prionopelta* is a more derived genus than *Amblyopone,* because of its
large colony size, specialized morphology and behavior of queens and workers, and the
occurrence of age polyethism. However, recent studies of *Amblyopone* revealed the occur-
rence of a clear age polyethism also in *A. silvestrii* (MASUKO, 1996) and *A. reclinata* Mayr,
1879 (ITO, unpubl.), an elegant recruitment system for prey retrieval and trophic egg laying
by virgin workers in *A. reclinata* (ITO, 1993a, unpubl.). Our study showed that a queen ret-
inue was not found in *P. kraepelini* and that the queen performed LHF as in *A. silvestrii.*
Colony size and other life history characteristics may affect the evolution of behavioral ele-
ments found in the two amblyoponine genera, rather than phylogenetic constraints.

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REFERENCES

BOURKE, A.F.G. & N.R. FRANKS (1995) – Social Evolution in Ants. Princeton University Press,
    Princeton : 529 pp.

BROWN, W.L. (1960) – Contribution toward a reclassification of the Formicidae. III Tribe
    Amblyoponini (Hymenoptera). *Bull. Mus. Comp. Zool. Harv. Univ.,* **122** : 145-230.

HASHIMOTO, Y. (1996) – Skeletomuscular modifications associated with the formation of an addi-
    tional petiole on the anterior abdominal segments in aculeate Hymenoptera. *Jap. J. Ent.,* **64** : 340-
    356.

HÖLLDOBLER, B. & E.O. WILSON (1986) – Ecology and behavior of the primitive cryptobiotic ant
    Prionopelta amabilis (Hymenoptera : Formicidae). *Insectes soc.,* **33** : 45-58.

HÖLLDOBLER, B., M. OBERMAYER & E.O. WILSON (1992) – Communication in the primitive crypto-
    biotic ant *Prionopelta amabilis* (Hymenoptera : Formicidae). *J. Comp. Physiol.,* **170A** : 9-16.

ITO, F. (1993a) – Observation of group recruitment to prey in a primitive ponerine ant *Amblyopone*
    sp. (*reclinata* group). *Insectes soc.,* **40** : 163-167.

ITO, F. (1993b) – Social organization in a primitive ponerine ant : queenless reproduction, dominance hier-
    archy and functional polygyny in *Amblyopone* sp. (*reclinata* group). *J. Nat. Hist.,* **27** : 1315-1324.

MASUKO, K. (1986) – Larval hemolymph feeding : a nondestructive parental cannibalism in the primitive ant *Amblyopone silvestrii* Wheeler (Hymenoptera : Formicidae). *Behav. Ecol. Sociobiol.,* **19** : 249-255.

MASUKO, K. (1989) – Larval hemolymph feeding in the ant *Leptanilla japonica* by use of a specialized duct organ, the «larval hemolymph tap» (Hymenoptera : Formicidae). *Behav. Ecol. Sociobiol.,* **24** : 127-132.

MASUKO, K. (1996) – Temporal division of labor among workers in the ponerine ant, *Amblyopone silvestrii* (Hymenoptera : Formicidae). *Sociobiology,* **28** : 131-151.

PEETERS, C., B. HÖLLDOBLER, M. MOFFETT & T.M. MUSTHAK ALI (1994) – «Wall-papering» and elaborate nest architecture in the ponerine ant *Harpegnathos saltator. Insectes soc.,* **41** : 211-218.

SCHÖNITZER, K., H. DOTT & R.R. MELZER (1996) – The antenna cleaner gland in *Messor rufitarsis* (Hymenoptera, Formicidae). *Tissue & Cell,* **28** : 107-113.

TRANIELLO, J.F.A. (1982) – Population structure and social organization in the primitive ant *Amblyopone pallipes* (Hymenoptera : Formicidae). *Psyche,* **89** : 65-80.

SHORT NOTE

IS THE WOODMOUSE (*APODEMUS SYLVATICUS*) OF SICILY A DISTINCT SPECIES?

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Key words : *Apodemus sylvaticus,* Mitochondrial DNA, Taxonomy, Biogeography, Sicily.

The Sicilian population of *Apodemus sylvaticus* (L., 1758) was initially considered a separate subspecies *A. s. dichrurus* Rafinesque, 1814. Two « morphs », one occurring around Palermo and the other near the Etna (1) were subsequently designated as subspecies (2). The Sicilian woodmouse is now considered a distinct species, *A. dichrurus* Von Lehmann & Schaeffer, 1976, based on its higher alkaline phosphatase activity (3). To evaluate the specific status of *A. dichrurus*, we have now studied mtDNA variation amongst 85 individuals from various populations in the Western Mediterranean area (Fig. 1).
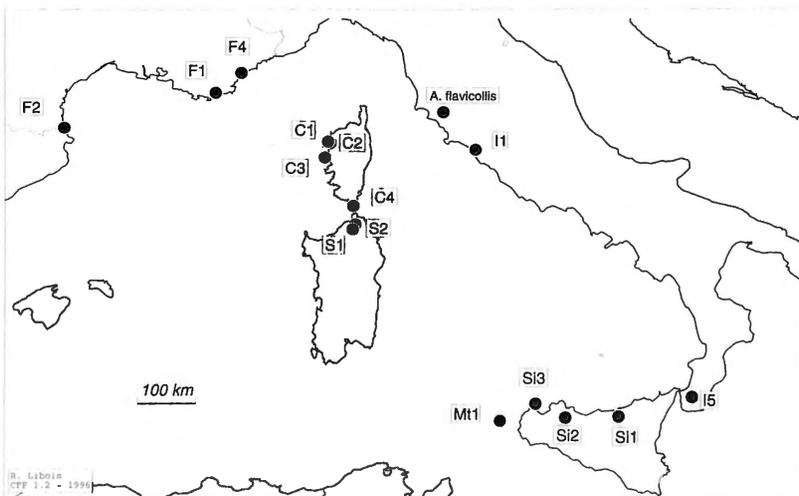

Fig. 1. – Geographic distribution of the sampling localities of *Apodemus*. F1: Cap Lardier*; F2: Banyuls/Mer*; F4: Estérel (Mt Vinaigre)*; I1: Tarquinia (Latium)*; I5: Gambarie (Calabria)*; C1: Fango*; C2: Fango (mouth)*; C3: Chiuni*; C4: Bonifacio; S1: Pietru*; S2: San Antonio*; Si1: Ficuzza; Si2: Grateri; Si3: St Vito lo Capo; Mt1: Marettimo; *A. flavicollis:* Grosseto (Tuscania). The localities with an * are the same as in Michaux *et al.* (1996) and are numbered in accordance.

Mitochondrial DNA was isolated from fresh tissue and digested with restriction endonucleases Hae III and Rsa I. The resulting fragments were separated in 4% PAA gels and subsequently silver stained (for further details: 4). The restriction patterns obtained were compared using the Nei & Li index (5) and a neighbour-joining tree (TREECON 1.2; see 6), was built using one individual yellow-necked field mouse A. flavicollis (Melchior, 1834) from Grosseto (Italy) as an outgroup. A bootstrap analysis (1000 replicates) was also performed to check the robustness of the nodes.

From the 86 animals analysed, 45 different haplotypes were obtained. The neighbour-joining tree (Fig. 2) shows that the yellow-necked field mouse haplotype is well separated from all the woodmice ones, the node separating them being very robust (BP of 100 %). The mean level of the nucleotide sequence divergence between the two species is 5.9 %.

The woodmice are divided into three distinct clusters: one cluster contains all the animals from peninsular Italy, Corsica and Sardinia, a second one contains those trapped in France, and the third one is formed by all the Sicilian samples. The mean level of genetic divergence between these three groups is quite high (between 2.6 and 3.8%), and this separation is very well supported with bootstrap values of 92 and 97%. We can therefore consider that the Sicilian woodmice constitute a third mtDNA lineage. In contrast, the intra-group divergence is very low: 1.4, 0.9 and 1.2 respectively, and of the same order of magnitude as that observed using the same technique in woodmice populations of Northern Europe (p ≈ 1%) (4, 7, 8) and in other rodent species (9, 10, 11). On the other hand, the differences in the values observed between the three woodmice groups of haplotypes are similar to those differences observed between subspecies of Mus domesticus L. 1758 (≈ 4 %) (10, 11). From a morphological point of view, the Sicilian mice differ only slightly from those of Sardinia or of Peninsular Italy; these differences being less than those found between the Italian and the French populations (12, 13) which are not separated at a specific level. Furthermore, fertile hybrids between animals from Sicily and either Italy or Germany have been obtained in captivity (3). For all these reasons we propose retention of the distinction between the Sicilian woodmouse and the other west European woodmouse populations only at the subspecific level (A. sylvaticus dichrurus).

Nevertheless, the mtDNA differences suggest that the Sicilian woodmice became isolated from the other groups about 750 000 years ago (using the calibration of Wilson et al.: 14). It is now well established from archaeozoological data (15), that the presence of the woodmouse in Sicily is the consequence of a Holocene anthropogenic introduction. It is also well known that Sicily has been invaded during the Holocene by numerous human groups from different geographic areas, notably Asia Minor and Greece (16). Our data suggest that the origin of the Sicilian woodmouse is not Peninsular Italy or Western Europe. This seems rather surprising since the strait of Messinia is only 3 km wide. Notwithstanding, it should be interesting to compare mtDNA variation between the Sicilian populations and those living in North Africa and in the eastern part of the Mediterranean basin.

Fig. 2. – Neighbour-joining tree illustrating the distribution of 45 mtDNA haplotypes. The nucleotide sequence divergence has been calculated according to Nei and Li (1979). The numbers near the main nodes are the bootstrap values obtained after 1000 repetitions. The restriction pattern code (in brackets) and the locality of origin define each branch.

REFERENCES

1. HAGEN, B. (1954) – Zur Kleinsäugerfauna Siziliens. *Bonn. Zool. Beitr.*, **1-2**: 1-15.

2. VON LEHMANN, E. & H.E. SCHAEFFER (1973) – Untersuchungen von Waldmäusen (*Apodemus sylvaticus*) und Gelbhalsmäusen (*Apodemus flavicollis*) in Kalabrien und Sizilien. *Suppl. Ric. Biol. Selvaggina, Lab. Zool. appl. Caccia, Bologna,* **6**: 175-184.

3. VON LEHMANN, E. & H.E. SCHAEFFER (1976) – Kreuzungsergebnisse mit Waldmäusen (*Apodemus*) der Insel Sizilien., *Säugertierkdl. Mitt.,* **24**: 180-184.

4. MICHAUX, J., M.-G. FILIPPUCCI, R.M. LIBOIS, R. MATAGNE & R. FONS (1996) – Biogeography and taxonomy of the wood mouse (*Apodemus sylvaticus*) in the Tyrrhenian region: enzymatic variations and mitochondrial DNA restriction patterns analysis. *Heredity,* **76** (3): 267-277 .

5. NEI, M. & W.-H. LI (1979) – Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci. USA,* **76**: 5269-5273.

6. VAN DE PEER, Y. & R. DE WACHTER (1996) – Construction of evolutionary distance trees with TREECON for Windows: accounting for variation in nucleotide substitution rate among sites. *Comput. Applic. Biosci,* **13**: 227-230.

7. TEGELSTRÖM, H. & M. JAAROLA (1989) – Genetic divergence in mitochondrial DNA between the wood mouse (*Apodemus sylvaticus*) and the yellow necked mouse (*A. flavicollis*). *Hereditas,* **111**: 49-60.

8. VAN ROMPAEY, J. (1989) – *Variatie en differentiatie van mitochondrial DNA in* Clethrionomys glareolus *(Schreber, 1780),* Apodemus sylvaticus *(Linnaeus, 1758) en* Apodemus flavicollis *(Melchior, 1834) (Mammalia, Rodentia) in West Europa.* Ph.D Thesis. University of Antwerpen, (166 pp).

9. BROWN, G. & M. SIMPSON (1981) – Intra- and interspecific variation of the mitochondrial genome in *Rattus rattus* and *Rattus norvegicus*: restriction enzyme analysis of variant mitochondrial DNA molecules and their evolutionary relationships. *Genetics,* **97**: 125-143.

10. FERRIS, S.D., R.D. SAGE, C.M. HUANG, J.T. NIELSEN, U. RITTE, U. & A.C. WILSON, (1983a) – Flow of mitochondrial DNA accross a species boundary. *Proc. Natl. Acad. Sci. USA,* **80**: 2290-2294.

11. FERRIS, S.D., R.D. SAGE, E.M. PRAGER., U. RITTE, & A.C WILSON (1983b) – Mitochondrial DNA evolution in mice. *Genetics,* **105**: 681-721.

12. SARA, M. & G. CASAMENTO (1996) – Morphometrics of the wood mouse (*Apodemus sylvaticus, Mammalia, Rodentia*) in the Mediterranean. *Boll. Zool.,* **62** (3): 313-320.

13. MICHAUX, J.R., R. LIBOIS & R. FONS (1996b) – Différenciation génétique et morphologique du mulot (*Apodemus sylvaticus*), dans le bassin méditerranéen occidental. *Vie et Milieu,* **46** (3/4): 193-203.

14. WILSON, A.C., R.L. CANN, S.M. CARR, M. GEORGE, U.B. GYLLENSTEIN, K.M. HELM-BYCHOWSKI, R.G. HIGUSHI, S.R., PALUMBI, E.M.M. PRAGER, R.D. SAGE & M. STONEKING (1985) – Mitochondrial DNA and two perspectives on evolutionary genetics. *Biol. J. Linn. Soc.,* **26**: 375-400.

15. TAGLIACOZZO, A. (1993) – Archeozoologia della Grotta dell'Uzzo. *Suppl. Boll. Paleoetn. it.,* **84** (2): 1-278.

16. TUSA, S (1993) – La Sicilia nella Preistoria. (2nd edn). Palermo, Sicilia: Sellerio.

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