EXPLOITING THE BIOLOGY OF URBAN MOSQUITOES FOR THEIR CONTROL

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Abstract - The phenomenon of autogeny has been well known in the *Culex pipiens* complex which consists of several physiological forms. Females of the autogenous form of *Cx. pipiens* are able to produce the first egg batch without a previous blood meal, whereas anautogenous females need a blood-meal for egg production. Autogeny is genetically determined, however the expression of autogeny is influenced by environmental factors such as larval nutrition, isolation of the breeding site or photoperiod. The autogenous females have to carry over reserves for the production of eggs from the larval stage. The fat body built up by the larva serves the female as source for the yolk production. The condition of food supply for the larvae and the degree of isolation of the breeding site affects the expression of autogeny in the adult. The autogenous form breeds usually below ground in enclosed spaces such as septic tanks and flooded basements in water bodies with a high degree of organic materials. In the northern part of the Palaearctic Region only the autogenous *Culex* populations bite man and represent the nuisance species. In contrast, the females of the anautogenous variant feed on birds and breed predominantly above ground in unpolluted water. A control strategy against *Cx. pipiens* should aim solely at the autogenous variant in order to have a target-orientated control operation against the nuisance species. In our study we investigated the biology of the sympatrically occurring variants, the degree of autogeny and finally we developed a monitoring system which enables us to differentiate between both variants. Besides rearing the various populations for proving the autogeny, we studied the genetic differentiation between the two variants by protein electrophoresis. This technique allows a quick identification of the autogenous variants to implement the control.
Key words - Autogeny, *Culex*, sympatric variants, electrophoresis

INTRODUCTION

The *Culex pipiens* complex consists of several forms, races, physiological variants or biotypes. Conventionally specimens of this complex from warm areas are designated as *Cx. quinquefasciatus* (Say) and from cooler areas as *Cx. pipiens* (L). Several morphological differences between these forms have been described e.g. morphology of the aedeagus, wing venation, color, scale pattern, siphonal index and branching of the siphonal tufts, as well as the lateral and transsutural setae (Barr, 1982). However, the only reliable diagnostic character to differentiate between these two forms is the phallosome of the male genitalia. The tips of the dorsal arms of the phallosome are thin and pointed in *Cx. quinquefasciatus* and thick and blunt in *Cx. pipiens*. Despite these differences there seems to be no genetic or cytological barrier which could prevent interbreeding. When hybridisation of the two forms occurs it results in specimens with intermediate characters e.g. in the male genitalia. However, incompatibility can especially be produced by a rickettsial endosymbiont *Wolbachia* spp. (Yen and Barr, 1973). Incompatibility ensues from the death of the sperm nucleus in an incompatible egg cytoplasm before karyogamy occurs (Rai, 1996).

In Europe, Ficalbi (1890) described for the first time two distinct forms of *Cx. pipiens* which differ in their biting behavior. One form which bites man and a second one which does not. Roubaud (1929), de Boissezon (1929) and Huff (1929) characterized the two forms of *Cx. pipiens* more precisely. One form mates only when much space is available for a mating swarm, requires a blood meal for the maturation of the egg batch, usually bites only birds, breeds mainly above ground and diapauses during winter. This form is taken as the typical form *Cx. pipiens* biotype *pipiens*. The second form is able to mate in small cages and does not require an obligatory blood meal for the maturation of the first egg batch. These characteristics are designated as stenogamy and autogeny, respectively. The name *Cx. pipiens* biotype

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molestus is settled on for this form. After laying the first egg-batch the females feed on man to use the blood for the increase of eggs per batch. It does not diapause during winter and usually breeds below ground in breeding sites with limited access to the outside such as septic tanks, flooded basements, cisterns. They are usually highly contaminated with organic material and are not exposed to frost. The autogenous females have to carry over reserves from the larval stage for the production of eggs. The fat body built up by the larva serves the female as a source for yolk production. Consequently newly emerged autogenous females have a higher body weight than anautogenous females.

Autogeny is genetically determined and regulated by alleles at a locus on the third chromosome as well as by a locus on the sex chromosome. The expression of autogeny is influenced by environmental factors such as restricted access to the breeding site, larval nutrition or photoperiod. Due to the selection pressure in these typical underground breeding sites it is likely that most matings in such places are between individuals which carry the genes for autogeny. Especially the condition of food supply for the larvae, the non-availability of hosts for a blood meal and the limited space for mating affects the expression of autogeny in the adult.

When females of *Cx. pipiens* enter a habitat with restricted access (e.g. cisterns, flooded basements or cess pools) where it is not likely to find a host for the blood meal the offspring will perish unless they can produce eggs autogenously by carrying the reserves for the production of eggs from the larval to the adult stage when larvae develop in breeding sites rich in nutrition. Furthermore, the selection will be rigourous for mating in restricted areas. Diapausing is no longer necessary because of the suitable microclimate in these underground habitats. Because suitable breeding sites for the autogenous and stenogamous form are man-made and closely associated with man, it is advantageous to bite man instead of birds. Especially in urban areas the autogenous form is a nuisance for man. In rural areas the anautogenous and ornithophile *Cx. pipiens* biotype *pipiens* is more abundant because natural breeding sites above ground are frequent and birds as hosts are also easily available.

Spielman (1964) postulated that *Cx. pipiens* biotype *pipiens* and *Cx. pipiens* biotype *molestus* are specifically distinct. He found that autogenous females gave rise almost exclusively to autogenous daughters and anautogenous females to anautogenous daughters. However, Barr (1982) states that despite the restricted gene flow between the two forms a significant amount of gene interchange can be assumed. There are many attempts to distinguish between the two forms by morphological characters (Marshall, 1938). The colour of the autogenous form could be lighter and the males could have shorter palps. However, these characters are either too subjective or too variable. So far the best distinction between the man-biting autogenous and the ornithophilic anautogenous form can be made on biological characters. The offspring of a given population is reared in small cages without a source for a blood meal and checked for autogeny.

In our study we have investigated the occurrence and distribution of the two forms in the Upper Rhine Valley and looked for possibilities to characterize the various populations by biochemical methods. It is of interest whether both sympatrically occurring forms are reproductive isolated and represent distinct metapopulations. If this is reflected by genetic differentiation it is likely to find genetic markers (heritable traits) for each form. The overall goal is to define the occurrence and distribution of *Cx. p.* biotype *molestus* which is the man-biting form - to allow the design of a cost-efficient control strategy. For this purpose, a population genetic study by means of enzyme electrophoresis was conducted in the KABS laboratories. This should allow a quick determination of the man-biting populations for a straightforward implementation of control tools.

MATERIALS AND METHODS

Ecological studies. In Southwest Germany samples of mosquitoes were taken from 50 different breeding sites of three geographically separated locations (about 120 km apart from each other). The breeding sites were selected according to their different degrees of access to the outside (above ground and underground) as well as contamination with organic material (see Table 1). From each breeding site at least 50 to 200 larvae were transferred in water deriving from the breeding site to rearing vessels of 300 ml (each contained about 50 larvae) and kept in small cages of 30 x 30 x 50 cm. The pupae were transferred to emerging vessels filled with tap water and the number of emerging adults were daily recorded in order to be able to compare the number of emerging adults and the number of egg rafts layed by autogenous females. The percentage of autogenous *Culex* populations of each different breeding site and the number of females being autogenous of each population were determined. The sympatrically occurring *Cx. torrentium* and *Cx. pipiens pipiens* have been seperated by their hypopygia. In the course of this study the mixture of both species can cause problems by the biochemical differentiation. A careful evaluation of the population structure is therefore necessary.

Abiotic parameters of the breeding site have been determined such as the conductivity, pH, content of ammonium, nitrite, nitrate, phosphate, chlorine and of organic materials. The latter have been characterized by determining the biological oxygen demand (BOD) which was needed to oxidize the organic subtances at 20° C in a dark chamber within two days (BOD_2). In this study water from six rainwater containers (with unautogenous populations) and 6 subterranean cess pools have been infestigated.

Protein electrophoresis. The populations of the above mentioned regions were further processed by biochemical treatment to characterize their isozyme patterns. It was important to involve populations from regions which are apart and isolated. This ensured that the results will not be influenced by geographic variation of the two forms of *Cx. pipiens*.

Electrophoresis was conducted in horizontal 1% agarose gels in a Multiphor II electrophoresis unit (Pharmacia). Electrophoretic conditions and staining recipes followed mainly Harris and Hopkinson (1976) and were adapted to mosquito specific requirements. 20 enzymes have been tested and eleven of them, representing at least 18 presumptive genetic loci showed activity and satisfying banding patterns. These enzymes were: Aconitate hydratase (ACOH), Adenylate kinase (AK-1, AK-2), Esterases (substrate: 1-naphthylacetate) (EST-1, EST-2, EST-3), Glycerol-3-phosphate dehydrogenase (G3PDH), Glucosephosphate isomerase (GPI), Hexokinase (HK-1, HK-2, HK-3), Isocitric dehydrogenase (IDH-1, IDH-2), Malate dehydrogenase NAD dependent (MDH-1, MDH-2), Malate dehydrogenase NADP dependent (MDH(NADP)), Mannosephosphate dehydrogenase (MPI), Phosphoglucomutase (PGM).

RESULTS

Occurrence and distribution of *Cx. pipiens* biotypes. One hundred percent of the populations deriving from subterranean eutrophic water bodies and more than 90% of the deriving females of each population were autogenous (Table 1). Also the underground water bodies with a less limited access such as air shafts and water catch basins held a high percentage (44%) of autogenous populations, whereas the populations from water bodies above ground such as water barrels, buckets and garden ponds were less frequently autogenous (0-20%).

It could be shown that *Cx. pipiens* is tolerant to a wide range of abiotic parameters. However, all autogenous populations derived from breeding sites with a BOD_2 between 120-167.5 mg O_2/l (average: 148.3 mg O_2/l) whereas the BOD in the six breeding sites with anautogenous populations varied between 4.5 to 11.25 mg O_2/l (average: 7.1 mg O_2/l). The proportion of autogenous females from the populations of the latter mentioned water bodies amounted to only 1-5%.

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Table 1. Occurrence of autogenous *Cx. pipiens* populations depending on different habitats.

Habitat	Number of sites	Number of autoge-nous populations	Percentage of occurring autogenous populations
subterranean isolated cisterns/ cess pools	9	9	100%
underground airshafts/catch basins	9	4	44%
above ground water barrels	22	2	9%
buckets	5	1	20%
ponds	2	0	0%

Protein electrophoresis. Altogether, 13 gene loci showed polymorphism. It could be demonstrated that the degree of heterozygosity in *Cx. p.* biotype *pipiens* is considerably higher than in *Cx. p.* biotype *molestus.* In some enzyme systems *Cx. p.* biotype *pipiens* possesses additional alleles that do not occur in *Cx. p.* biotype *molestus*. So far it can be stated that the gene loci of MPI, PGM, AK-1, HK-1,2,3 showed significant differences in the allele frequencies. All four enzymes are suitable as genetic markers due to their evident allozyme differentiation (Figure 1). Another interesting aspect is the obviously different expression of esterase genes in both forms. *Cx. p.* biotype *pipiens* showed a rhich allozyme pattern, while many of the *Cx. p.* biotype *molestus* specimen had only two bands. It is likely that in *Cx. p. molestus* less esterase gene loci are expressed.

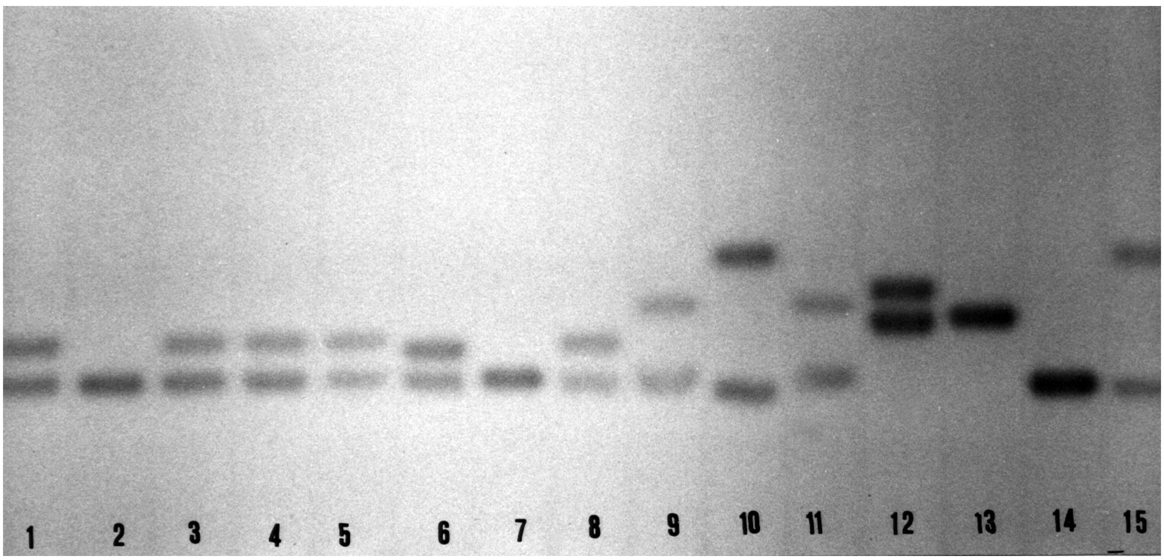

Figure 1. The enzyme system of MPI shows heterozygosity with two electromorphs in *Cx. pipines molestus* (runs 1-8 on the left) and six electromorphs in *Cx. pipiens pipiens* (runs 9-15 on the right).

DISCUSSION

Environmental factors lead to the expression of autogeny. The more restricted and polluted with organic materials the breeding sites are, the more is the likelyhood of the occurrence of autogenous *Culex pipiens* populations - producing females which bite humans. In Germany it could frequently be recognized that *Culex* populations which derive from water containers in garden areas do not cause nuisance

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problems despite of their numerous occurence. Obviously in this locations the biotype of *Culex pipiens* dominates which prefers birds as hosts for the blood meal. In contrast, in areas where subterranean anthropogenous breeding sites of *Culex* occurred complaints of the public about nuisance problems caused by *Culex* even during winter times increased during the last years. The reason for that is the increasing number of cisterns. The people are asked to collect rainwater for usage in the garden areas and to avoid a decreasing ground water level by draining the water into the canalization. Due to the increasing problem with *Culex pipiens*, this study was initiated to assess the distribution of autogenous *Culex* populations and to develop a straightforward control strategy. According to our results autogenous populations breed predominantly in septic tanks and cess pools in human settlements. Especially these populations cause serious nuisance problems. The question was how distinct these populations are. Our studies characterize *Cx. p* .biotype *pipiens* and *Cx. p.* biotype *molestus* populations in human settlements as extensively isolated forms concerning their reproduction with distinguished patterns of isozyms. So far our data indicate that the genetic distances between geographically separated populations of *Cx. p.* biotype *pipiens* are much smaller than the genetic distance between adjacent populations of *Cx. p.*biotype *pipiens* and *Cx. p.* biotype *molestus*, respectively. It can be assumed that both forms already represent subspecies due to their limited gene flow.

We have shown that it is possible to identify both forms by qualitative biochemical characters. Especially the enzymes MPI, PGM, AK-1, HK-1,2,3 seem to be suitable genetic markers to distinguish between both biotypes, the man-biting and the non-man-biting form. So far, enzyme electrophoresis seems to be suitable for an extensive inventory of the subspecies distribution. This allows a straightforward implementation of control operations against the man-biting mosquito instead of a random control of both forms. Thus the control is more efficient and the costs for the operation can be drastically reduced.

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