Distinguished Achievement Award Presentation at the 2002 Society of Vector Ecology Meeting

Physiological bases of mosquito ecology

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ABSTRACT: The research carried out during more than 30 years in the author's laboratory is briefly reviewed. Quantitative analyses of basic physiological processes, such as growth and development, digestion and excretion, oogenesis and fecundity, reserve synthesis and resulting flight-potentials of *Aedes aegypti* were summarized and compared with several other mosquito species, particularly with *Anopheles*. These studies led to the recognition of distinctly different physiological strategies, for which the term "physiotype" has been coined, providing a basis for understanding the different ecotypes. ***Journal of Vector Ecology* 28(1): 1-11. 2003.**

Keyword Index: Reproduction, metabolism, flight, eco-physiology, strategies.

First and foremost, let me express my sincere gratitude to the Society for Vector Ecology for this prestigious Distinguished Achievement Award and for the opportunity to attend this meeting. It is good to see so many scholars of mosquito biology together again. I also appreciate the opportunity to present a short overview of our work that has been carried out during the last three decades, mainly in Switzerland. I will try to draw a general and very personal picture of some physiological aspects of mosquito life styles that may provide a background for a better understanding of ecological situations encountered in the field. For that purpose, I would like to point out the advantages of fully quantitative analytical procedures available in the laboratory and what we can learn from such studies. We worked in several fields of comparative physiology with different mosquito species.

My research began with studies of blood digestion in *Aedes aegypti* (Briegel and Lea 1975, 1979, Briegel 1981, 1983). We clarified the enzymatic and endocrine regulations of the processing of the blood meals (Graf and Briegel 1982, Hörler and Briegel 1995, 1997), and for the first time, achieved the purification of mosquito trypsin and characterized its synthetic pathway (Graf and Briegel 1985, 1989, Graf et al. 1986, 1998). Because there is no digestion without excretion, we also quantitatively studied the excretory processes in great detail (Briegel 1975, 1980a, b, 1986, Briegel et al. 1979, von Dungern and Briegel 2001a,b). This led us to an understanding of stoichiometric relationships and principles governing the metabolic events during the reproductive cycles of *Ae. aegypti* (Briegel 1985, 1990a) and *Anopheles* species (Briegel and Rezzonico 1985, Briegel and Hörler 1993, Klowden and Briegel 1994). Once we had quantified blood ingestion, protein digestion, and excretion of the catabolites, we had a metabolic background for entering the field of reproductive physiology and oogenesis (Briegel 1985, 1986, Lea et al. 1978, Briegel et al. 2002). We then expanded to larval development (Timmermann and Briegel 1996, 1998, 1999) and to other mosquito species, and we finally became entangled in the flight potential and energetics of female mosquitoes (Briegel et al. 2001a,b, Briegel and Timmermann 2001). Using flight mills, we were able to record the flight distances, the patterns of flight activity, and the utilization of reserves in comparison to pre-flight conditions and to those that had not flown. During all phases of our research, I had always intended to address and answer basic questions about the mosquito and its actual behavior in the field and/or ecological adaptations of certain species. I will select a few examples and results to put parts of the physiological mosaic together to reveal a picture of the ecophysiology of a species. In doing so, distinct species-specific strategies could be identified.

The larval period of mosquitoes is a primary target of all control methods. Therefore, we studied their growth and development, their proteolytic enzymes, and their synthesis of reserves (Timmermann and Briegel 1996,

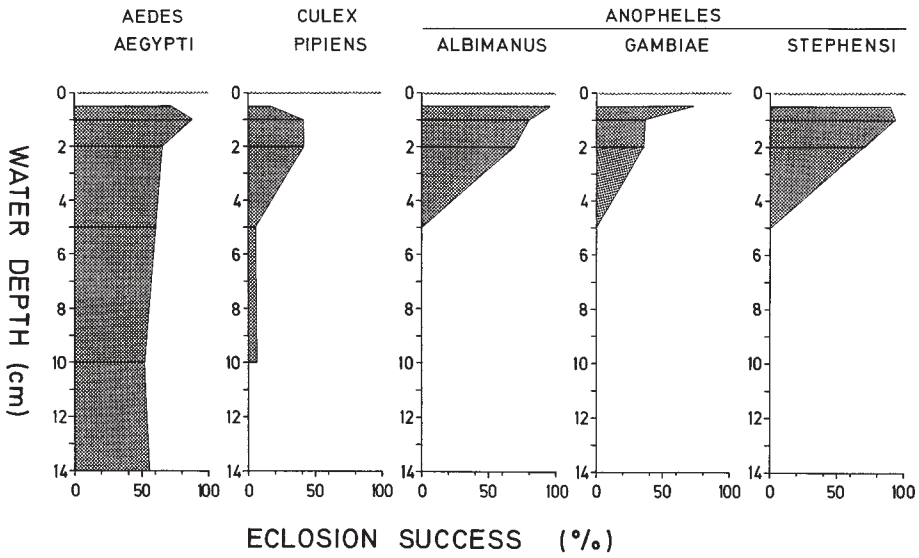

Figure 1. Effect of depth of water in the larval rearing containers in five mosquito species. These containers were rat cages (22x40 cm, 15 cm high) filled with water to depths of 0.5, 1, 2, 5, and 14 cm, each with 200 larvae. Total eclosion of imagoes is expressed as a percent of newly hatched larvae. Optimal development was always at water depths of 0.5 to 1 cm.

1998, 1999, Hörler and Briegel 1995, 1997). Different feeding mechanisms are commonly recognized among various groups (Dahl 1993). We quantitatively demonstrated that mosquito larvae could utilize only a narrow water segment between the water surface and the deeper zones (Figure 1, Timmermann and Briegel, unpublished data). This observation provided a general understanding of mosquitoes that usually breed in shallow water ponds, always close to the surface or to the margins. Therefore, our interests were centered around the nutritional aspects. It turned out that mosquito larvae definitely require food of animal origin in addition to plant debris (Timmermann and Briegel 1996) in order to meet their needs for the essential poly-unsaturated fatty acids that apparently do not occur in plant material, as already had been shown by Dadd (1981, 1983). Mosquito larvae feed continuously, similar to caterpillars. Some elegant endocrine mechanisms regulating molting and metamorphosis in Lepidoptera have been revealed (Nijhout 1975), but to our surprise, mosquito larvae were different. While caterpillars are able to adapt their molts to their nutritional status, even through supernumerary instars, this is impossible in mosquitoes. They are programmed for four larval instars, and the environmental, mainly dietary conditions, determine their final body size. We found an absolute value for each instar in the diameter of their head capsule (confirming Dyar's rule with a factor of 1.1), whereas the thorax diameter indicates the biomass acquired by the larva (Timmermann and Briegel 1998). During the fourth and last instar, 80-90% of the growth and biosynthesis takes place; if not enough is accumulated, the larvae can wait for 2-3 weeks until they finally pupate or die. If they pupate they give rise to small or large imagoes, while the dead larvae provide additional food for their sisters and brothers. I say sisters and brothers, because during the fourth instar the sexual differences in growth and biosynthesis become expressed (Timmermann and Briegel 1999). Because most mosquito larvae live in stiff opposition between feeding in the water column or on the bottom and breathing at the surface, the biosphere accessible to them is often confined to only a few centimeters, particularly in the Anophelines, which lack a siphon and spend most of their lives at the surface (Timmermann and Briegel 1993). These constraints are reflected in variable imaginal body sizes and their teneral reserves that in turn, strongly affect the life of the imagoes (Briegel 1990b, Figure 2).

After eclosion, insects enter a shorter or longer "maturation period," the teneral phase. In mosquitoes this lasts about one day. This is a very important period, because so many covert effects take place at all levels: anatomical differentiation, behavioral maturation, the hormonal system, the digestive system, vitellogenesis, and also the flight muscles. All require their time before the true nature of the mosquito and its blood-sucking habits become manifested. Among all these aspects, the quantity of teneral reserves "inherited" from the larva are crucial (Figure 2, Briegel 1990a, b).

When studying the physiology of any insect, there is one very important parameter to consider that is obvious but often neglected: body size. We had initiated rigid evaluations of mosquito body sizes in all our research. In insects, particularly in mosquitoes, weight determinations are often misleading except for the dry weights. Therefore, morphometric measures are always best, but for physiological considerations, volume and not linear dimensions are required (Schmidt-Nielsen 1984). Consequently, we decided for the simplest treatment, which is the cubic value of the wing length, or some other length of the exoskeleton. In this way, body size is a dependable measure for these reserve conditions that follow significant isometric or allometric relationships, although with species-specific slopes and extent (Briegel 1990a, b; and Figure 2). We routinely produced mosquitoes covering the whole spectrum of possible sizes in several species (Briegel 1990a, b). This is easily accomplished by manipulating the food supplies and/or the population densities during the larval period. Different reproductive strategies are recognized. Females may or may not profit from the larval reserve accumulations, and accordingly they seek additional sources. Of course, blood from vertebrates is a valuable, although a difficult and dangerous source for the additional protein required. This is the central issue of hematophagy and of most investigations on mosquito physiology, besides the problems of pathogen transmission. However, there is an equally important and more variable story involving the synthesis and reserves of lipids, their needs for yolk production, and their energetic significance.

Total protein always follows a highly significant and linear correlation with body size because protein at this stage represents mainly a structural component. Lipids on the other hand can follow linear correlations with body size, equal to protein or much higher, or they show exponential correlations of steep or flat inclinations (Briegel 1990a, b). To compensate for low teneral lipids, females may seek sugar sources, which then enter the lipogenetic pathways, in most cases before blood meals are added (Briegel et al. 2001a, b). Hormonal factors suppress glycogen synthesis, thus favoring lipogenesis to some degree of obesity (Van Handel 1965, Lea and Van Handel 1970). The amount of sugar ingested before a blood meal and the extent of subsequent reserve build-up are detrimental for the survival of the females (Figure 3). Sugar solutions provided in various concentrations clearly affect the survival of a population. As will be shown later, the amount of lipids accumulated until the time of a blood meal largely contributes to the reproductive potential of a female mosquito. Selected examples shall demonstrate the metabolic strategies recognized so far; autogenous females and males are not considered at this point.

First, let's consider the classic case of *Ae. aegypti*, which has been thoroughly studied in so many respects. The linear regression for protein against body size and the exponential regression for lipid have been shown in Figure 2 with the isometric and positive allometric functions, respectively (Briegel 1990a). Within a few hours of eclosion, these females begin to take sugar if available, thus improving their teneral lipids. When deprived of sugar, they avidly take blood meals within one day and can survive, but not necessarily reproduce, unless they are of large body size. Blood meals add a substantial amount of protein. In our experiments we always gave measured blood meals by enema to each female (0.5–5 µl), followed by individual micro-analyses of intestinal enzyme activities, of the yolk components, and of the principal catabolites (Briegel 1985, 1986, 1990a). Figure 4 combines such measurements during a gonotrophic cycle of large *Ae. aegypti*. Figure 5 demonstrates the perfect synchronization amongst the various processes: yolk volume parallels the appearance of yolk protein and yolk lipid, whereas glycogen appears at the end, shortly before chorionation. Uric acid synthesis parallels urea formation, and both coincide with yolk deposition, as shown in Figure 4. All the events taking place in such a narrow sigmoid area (hatched area in Figure 4) point out the remarkable capability of the fat body cells to distribute the nitrogen units to synthetic mechanisms (vitellogenin) and to the catabolic mechanisms (uricotely, ureotely, and ammonotely). In addition, the carbohydrate units obtained through protein degradation are also converted to appropriate amounts of lipid synthesis. Hematin defecation marks the end of the digestion period and was shown to be regulated on a neuronal basis (Briegel and Lea, unpublished data, Van Handel and Klowden 1996). These measurements also allowed the determination of the extent of the utilization of blood for oogenesis in large and small females, and the establishment of complete protein budgets (Figure 6). As a rule, blood meal protein is always ingested in surplus of the need for vitellogenesis. In *Ae. aegypti*, roughly one-third of the human blood protein is utilized for synthesis of yolk protein, while two-thirds are deaminated and split equally for renewed lipid synthesis and for energy requirements. Ziegler and Ibrahim (2001) found a remarkable mechanism in which after a blood meal, yolk lipid is taken from the pre-blood meal lipid stores in the fat body, while the lipid synthesized from the blood protein is deposited into the fat body for the next gonotrophic cycle. In addition, we had found evidence for a threshold of one calorie for the female lipid content plus the blood meal

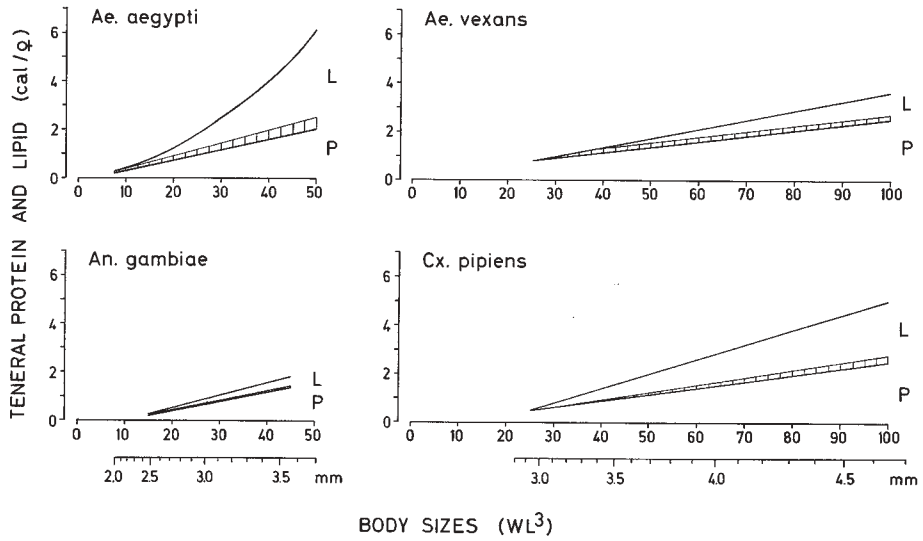

Figure 2. Teneral protein (P) and lipid (L) of four mosquito species, measured shortly after eclosion and plotted against body size. All possible body sizes measured as wing length (mm) and presented as cubic value (WL^3) were produced by manipulating larval densities and food supplies. Only the regression lines are shown. The small hatched segment represents the minimal amounts of lipid observed when females were starved to death, thus indicating the extent of lipids available for mobilization under extreme nutritive stress. (Data from Briegel 1990a, b; Briegel et al. 2001; and unpublished data).

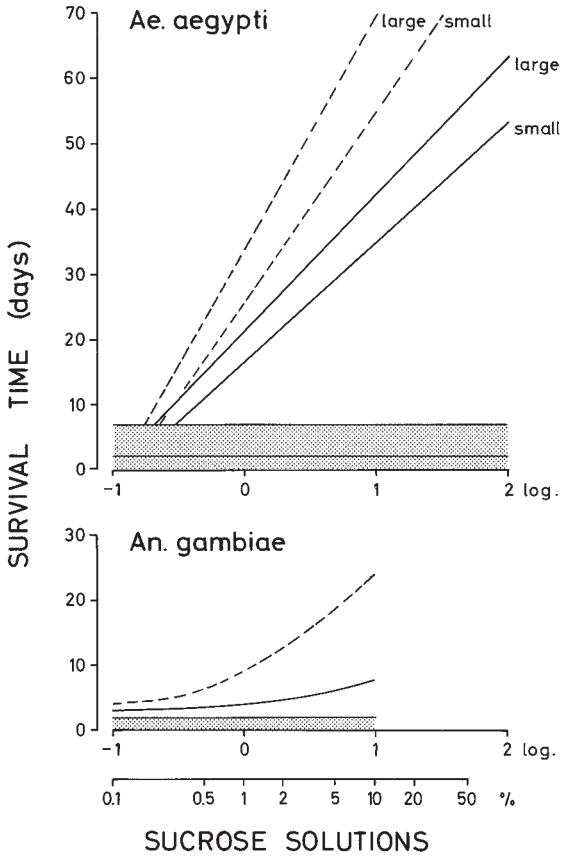

Figure 3. Effect of increasing concentrations of dietary sugar solutions on female survival for large and small *Ae. aegypti* and *An. gambiae*. The 50% (solid lines) and maximal (dashed lines) survival times are depicted as regressions, based on earlier data (Briegel et al. 2001a, b, and unpublished data). Survival under starvation, i.e. access to water only, is shown by gray shading for the cohorts tested.

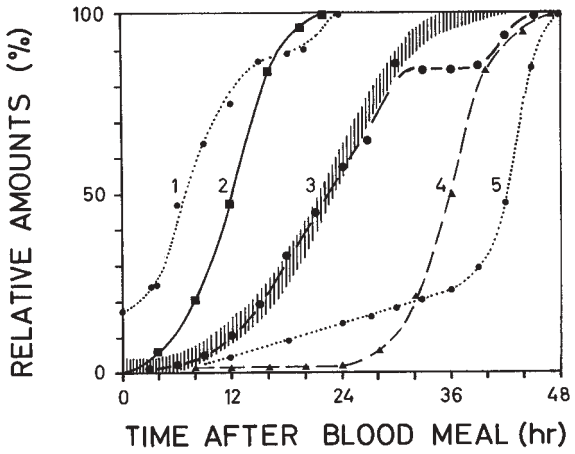

Figure 4. Temporal sequence of physiological processes during one gonotrophic cycle of *Ae. aegypti*, all values expressed as a percent of their respective maxima. 1: intestinal amino peptidase activity; 2: intestinal trypsin activity; 3: yolk volume (dots and line); the shaded area comprises uric acid and urea excretion, yolk protein and yolk lipid deposition into oocytes; 4: hematin defecation; 5: yolk glycogen deposition into oocytes. Arginase activity in the fat body closely follows the course of intestinal trypsin activity, and xanthin dehydrogenase (XDH), also localized in the fat body, parrallels intestinal leucine amino peptidase activity, both not shown for reasons of clarity. Arginase has a 10%, and XDH a 40% residual activity, because these two enzymes are active throughout life. (Data adopted from our earlier publications).

protein to be ingested, critical for initiation of oogenesis, and not just the amounts of blood meal intake (Briegel, unpublished data). Together, these observations explain that the teneral and/or sugar-derived lipid (Figure 7, bottom) is relevant for the reproductive processes once protein has been ingested. This also explains why small females are unable to mature a batch of eggs with their first blood meal on teneral reserves alone. When sugar-fed females obtained rat blood however, fecundity was doubled as a consequence of non-limiting isoleucine contents in rodent blood (Briegel 1985). Furthermore, there were reversed proportions between uric acid and urea excretion, depending on whether females had access to water (Figure 6: d versus w). In the three *Anopheles* species tested, however, yolk protein always remained below 20% of the blood meal protein.

To investigate the significance of the lipid reserves of sugar-fed female *Ae. aegypti*, we recently followed the lipid metabolism through 5-6 gonotrophic cycles in large and small females (Briegel et al. 2002). As the females became physiologically older, the more they withheld the lipid in their fat body instead of transferring it into oocytes. This surprising result provides an explanation for the steadily declining fecundity in older females; they become "fat old ladies." The physiological alterations within the fat body should be investigated to fully understand this "selfish" behavior of older *Ae. aegypti* females. Figure 7 also shows the development of strong flight potentials for *Ae. aegypti*. After eclosion it takes 2-3 days of sugar-feeding for females to reach their strongest flight potentials of >5000 m/night in 50% of the cohort.

Ae. vexans, a major nuisance in the holarctic region and known for its migratory flights (Clements 1999), is a different case. It is of larger size but considerably lower in teneral protein and lipids (Briegel et al. 2001a, Figure 2). How does it cope with its poor teneral reserve status? During the first week of imaginal life, female *Ae. vexans* must feed on sugar or otherwise die within 4-6 days. They can multiply their teneral lipid roughly 10 times during the first week, and up to 20 times during the second week (Figure 8). Once the lipids have been increased during their first week, females seek blood meals. After 5-6 d, up to 100-120 eggs may be matured per female. Before that, they generally do not take blood meals, but if these were forced, egg maturation failed. At the same time, we recorded the flight potentials of individual mosquitoes. Their reserves are routinely analyzed before and after flights and compared to fixed-wing controls kept 20 h motion-less. The data from such flight protocols (Figure 9 as one example) reveal the total flight distances in km, and the temporal pattern of flight activities. Non-stop flight segments of several hours are regularly observed, interrupted by periods of inactivity that we assume represent phases of reserve mobilizations from the abdominal fat body, the primary storage site, to the flight muscles. The flight potential is marginal during the first few days, but it develops from the second week onwards. Flights of up to 17 km within one night become possible, indicating typical migratory behavior (Figure 8). During such strong flights, up to 50% of the glycogen reserves are utilized together with a 7% disappearance of the lipids.

In conclusion, two phases are clearly recognized in this species: first fill up the fat body with lipids, for which no long flights are required, while at the same time the reproductive system requires a maturation period of similar duration as the flight muscles. Only afterwards, hunger for the missing protein develops and blood donors need to be found by migrations over long distances. Indeed, females attacking us in the field always carry remarkable lipid reserves that are considerably above their teneral values. Similar strategies were

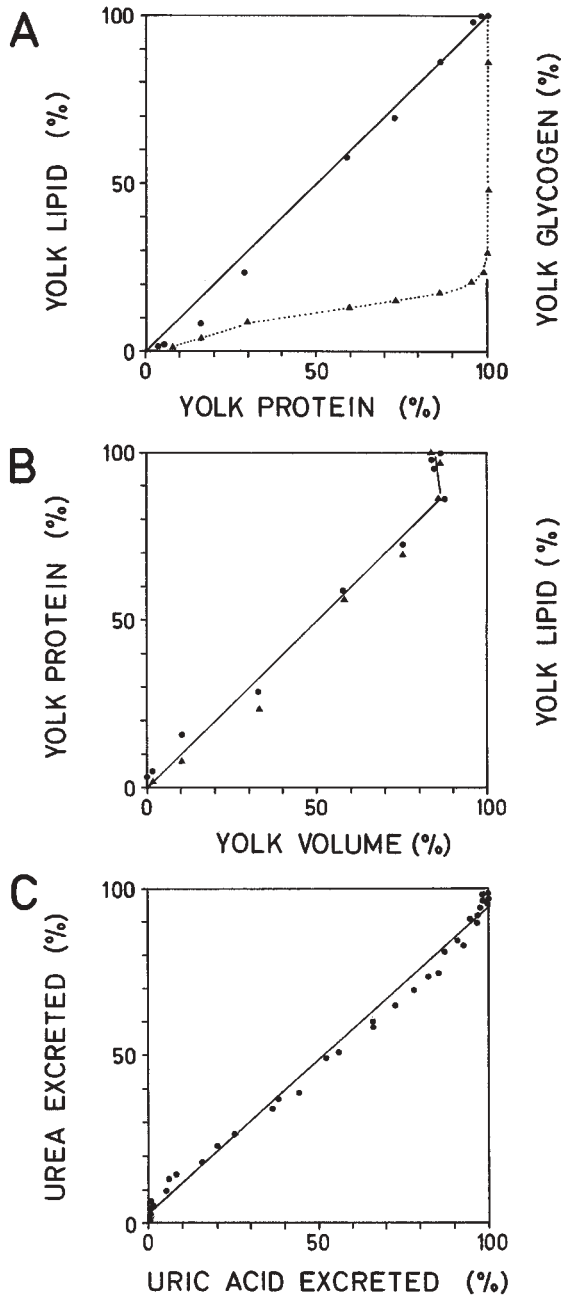

Figure 5. Demonstration of synchronous synthesis of yolk protein and yolk lipid (A), parallel to increasing yolk volumes (B); uric acid is synthesized in synchrony with urea formation (C), and both are synchronous with yolk protein and lipid deposition (compare Figure 3). Only glycogen synthesis occurs much later. The discontinuity of yolk protein and lipid formation at volumes of 90% (B) is caused by the elongation of the oocytes observed between 30-36 hr after blood meal (from Briegel et al. 2003).

described by Renshaw et al. (1995) for *Oc. cantan*s which resembles the *Ae. vexans* type, and were also found in *Ae. triseriatus* (Briegel unpublished data) and *Ae. albopictus* (Briegel and Timmermann 2001), as well as in *Oc. punctor* (Renshaw et al. (1995), the latter three resembling *Ae. aegypti*.

Still another situation is encountered in the Anophelini. As mentioned before, these are surface feeders and the air-water interface is of lower nutritional value than the bottom of a puddle with all its organic matter. *An. gambiae* has a smaller body size, and *An. albimanus* is similar to or even larger than *Ae. aegypti*, but because in both *Anopheles* species, protein and lipid follow isometric relationships, although on a much lower level, they might be called "skinny" (Briegel 1990b). Tropical *Anopheles* have evolved the following strategies and adaptations. First of all, they can successfully feed on blood donors within half a day of eclosion (Figure 7), often without extended sugar feeding. Indeed, their lipogenetic capabilities are lower than in Culicini. But appreciable flight potentials soon develop (Figure 7). Second, the blood meal protein is concentrated during feeding, comparable to aphids with their water (and sugar) removal, which in turn requires extended feeding times (Briegel and Rezzonico 1985) and is in line with their preference for biting at night when hosts are less active and less sensitive. This behavior allows blood meal volumes of up to 10-12 µl (or mg), as compared to usually 2-5 µl in *Aedes*. In addition, *An. albimanus* express a considerable constitutive trypsin activity in their midgut shortly after eclosion, which allows immediate digestion after blood ingestion, well before the inductive trypsin is synthesized a few hours later. Third, multiple blood meals can be taken at short intervals, even within a single night, occasionally leading to a daily oviposition rhythm (Hörler and Briegel 1995).

Furthermore, in *An. albimanus*, which are smaller than 3 mm wing length, the first blood meal is used for the synthesis of maternal lipid and protein reserves, and not for yolk, thus compensating for their low teneral amounts (Briegel 1990a). Synthesis of yolk components in such instances is achieved from subsequent blood meals, but the efficiency of blood protein utilization for yolk synthesis always remains <10% of the caloric input, much lower than in Culicini. In spite of these metabolic constraints, total fecundity of *An. albimanus* with 200-400 eggs per female within the first week of its imaginal life with daily blood meals is roughly equal, if not higher than the fecundity of about 290 eggs per female in *Ae. aegypti* during the same period.

Thus, I think the concept of gonotrophic cycles is questionable for *Anopheles* species. It appears that more or less rigid gonotrophic cycles may have evolved

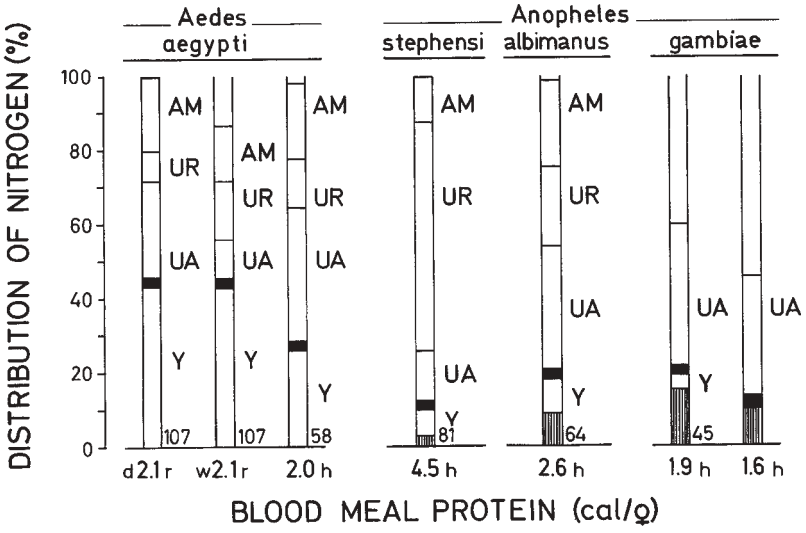

Figure 6. Nitrogen partitioning during a gonotrophic cycle of four mosquito species fed human blood (h). All females had access to sucrose before the blood meal. *Ae. aegypti* was also fed rodent blood (r), and furthermore were kept in dryness (d) or with access to drinking water (w). The black segments are for haematin which always accounts for 2-3%. Note the variable protein input by blood meal, caused by the body sizes and midgut volumes of the different *Anopheles* species; in *Ae. aegypti* blood meals were given by enema. The average number of eggs matured for each condition is added at the bottom of each bar. The segment with vertical shading represents the maternal, extraovarian protein deposit, synthesized from the blood meal before and/or together with the yolk protein. Further explanations see text. Abbreviations: Y for yolk, UA for uric acid, UR for urea, AM for ammonium plus amino nitrogen (combined). In *An. gambiae* the budget was not completed.

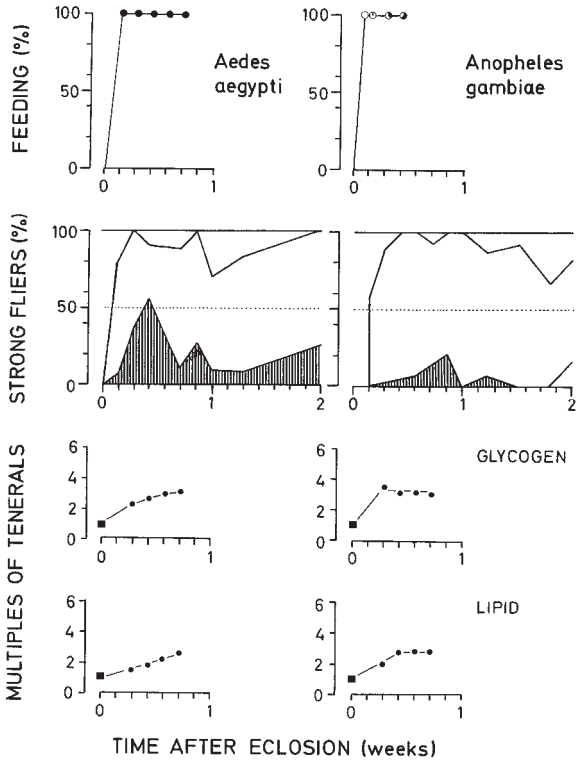

Figure 7. Comparison of reserve synthesis, feeding attempts and success, and flight performances between female *Ae. aegypti* and *An. gambiae*. For the synthesis of glycogen and lipid (bottom panel) the caloric values are expressed as multiples of the respective teneral values, that arbitrarily are given as 1. The feeding activity develops within 1 day of eclosion in both species (top panel), but successful oogenesis is delayed in *An. gambiae* (indicated by black segments within the circles). Flight performance in *Ae. aegypti* revealed two clearly visible segments: strong fliers (i.e. >5 km per night, dark shading) were encountered already by day 3 (over 50%), whereas the majority of the remaining females were average fliers (i.e. 1000-5000 m/night); the smallest segments were poor fliers. *An gambiae* are mostly average fliers; only at days 4, 5, 8, and 14 some very strong fliers (>5000m/night; 10-20%) were observed with up to 17 km/night.

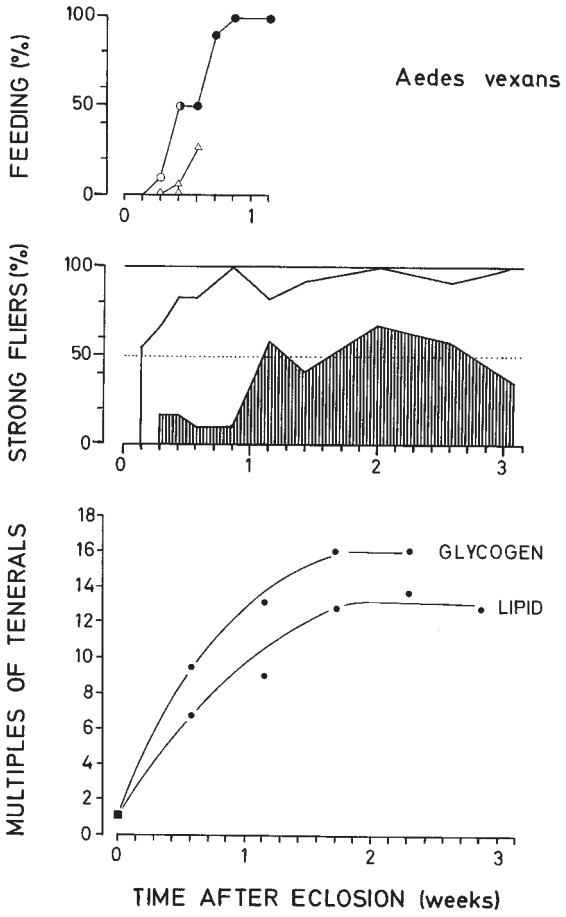

Figure 8. Feeding (top), flying (center), and reserve synthesis (bottom) in *Ae. vexans*, showing a different timing and extent of these events and therefore different strategies than in the other two species of the foregoing Figure. Note the tremendous amounts of reserves built up during the first two weeks of imaginal life (bottom panel). Feeding success followed by oogenesis requires roughly one week of development (top panel). Flight performances also develop slowly and gradually. Strong fliers (dark shading) reached up to 17 km per single night on the flight mill, but only after several days. In the top panel, triangles are for females that had access to water from eclosion, circles for sugar-fed females; the extent of black indicates 50% or 100% oogenesis in these female cohorts.

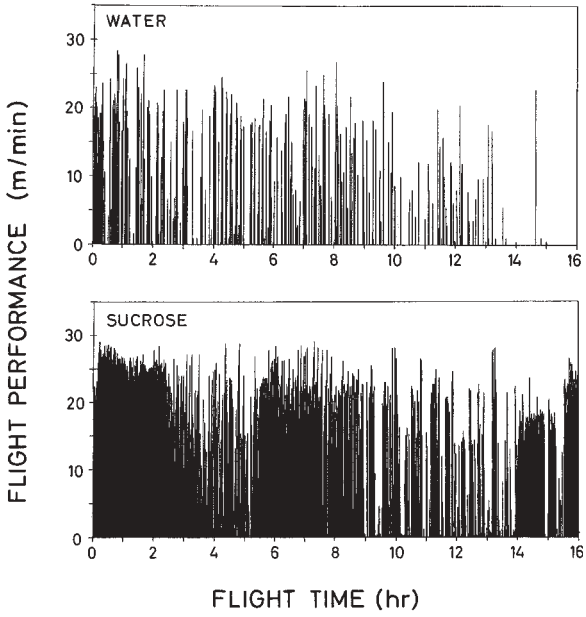

Figure 9. Two examples of flight mill protocols of 3-day-old female *Ae. aegypti* with access to water (top) or sugar (bottom). The total flight distances flown during 16 hr were 0.9±0.8 km (N=14) and 5.7±3.0 km (N=16) per female, respectively.

predominately in the Culicini. If true, this suggestion bears considerable consequences for the hormonal regulations that have been established for *Aedes* or *Culex* (Lea 1975). The hormones might be the same ones among the two tribes (M.R. Brown, personal communication), but no cyclicity has to be maintained in the Anophelini.

Another interesting aspect was encountered during our flight studies with *An. gambiae* (Figure 7) or *An. albimanus*, both strong fliers. We always found substantial reductions of their pre-blood meal lipid contents during the flight periods. The metabolic basis for lipid oxidation by flight muscles unfortunately has not been investigated in these insects. An alternative possibility would be to test for proline oxidation, which depends on the lipid reserves, as was found earlier for *Glossina* (Bursell et al. 1974, Langley 1977).

For pathogen-carrying females, such as those infected with *Plasmodium*, our results bear epidemiological consequences, that suggest much higher vector potentials than assumed so far. To fully ascertain such epidemiological consequences in *Anopheles*, experiments should be extended for at least the duration of the extrinsic incubation time of *Plasmodium*.

We are considering an evolutionary interpretation that the physiological and endocrine principles governing the discrete gonotrophic cycles in Culicines have evolved later, representing an apomorph status. I even dare to think that the possibility for acyclic reproduction in tropical *Anopheles* as a plesiomorph status might contribute to or explain the fact that *Plasmodium* has adopted Anophelini as their vectors, and not the Culicini.

In conclusion, I would like to convey a few personal remarks. Some entomologists have objected to issues raised from our physiological laboratory investigations versus ecological and epidemiological field studies. I never claimed that our laboratory-based results with controlled and constant conditions would represent the actual life of a given vector in its natural environment, and I am aware of many additional parameters in the field that equally govern mosquito ecology. What I have always tried to provide, however, was a valid and largely quantitative background, and to present a framework against which ecologists could seek additional possibilities or interpretations of their findings. In the laboratory I think we could stretch that frame to the extremes, while other parameters were kept constant, to enable a view on the full metabolic potential of a given species. I am also aware that a next generation of physiologists will have to take the next and more difficult steps to investigate such additional parameters. There remains a lot of work for future physiologists to do but all this is a challenging outlook for young entomologists. The hard core of this work ought to be done at the bench, much more than on computer screens, and then be combined with field studies.

The different strategies presented above reflect a biodiversity on the physiological level; there is no physiology of "the mosquito." Each group and even species has its own set of metabolic adaptations, required to fit its respective ecological niche. It will be interesting to unravel new and arrange alternative strategies among the many mosquito vector species. To ease comparative approaches along the lines presented here, I suggest a new term for these adaptations: the "physiotype," in analogy to the ecotypes recognized by ecologists.

As a final consideration, I believe that models created on computer screens are too simplistic and often misleading. Our knowledge is far too limited to feed into theoretical programs. One should not be afraid to ask about the secrets of the life of the insect by devising clever experiments. It is far more interesting, challenging, rewarding, and true. We have only started to understand merely five to six species a little better, out of the dozens of vector species and parasites they transmit. There remains an endless future for the field of basic physiology.

Acknowledgments

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