European Mosquito Bulletin, 12 (2002), 7-16.
Journal of the European Mosquito Control Association
ISSN1460-6127

Colonisation of the floodwater mosquito *Aedes vexans* (Meigen) (Diptera: Culicidae)

Roland Kuhn
Department of Zoology (2130 005), Johannes Gutenberg University, D 55099 Mainz, Germany.
Email: roku@oekologie.biologie.uni-mainz.de

Abstract

A method of laboratory colonisation of several European and North American populations of *Aedes vexans* is described. A colony from the Rhine River Valley, near Mainz, Germany, has been maintained in the laboratory for more than 150 cage generations. Another from the Queich River in the Rhine Valley of Southern Palatia, Germany has been maintained for 22 generations, and a colony from the Ticino River delta of Switzerland has been maintained for 12 generations. Six North American strains, one each from the states of Minnesota, Arizona, Oregon and Utah, and the Canadian Provinces of Manitoba and Ontario, are in cage generations 52, 20, 19, 14, 41 and 15 respectively. Colonisation of different strains of this widespread pest mosquito opens up many research avenues.

Introduction

Aedes vexans (Meigen) is the most abundant floodwater mosquito in the Northern Hemisphere (O'Malley, 1990), where it is a serious blood-feeding pest of humans and animals. The ecology, disease vector status, and the control of this species has been the subject of numerous studies over the past century. Most of the major literature can be found in Horsfall (1955), Horsfall *et al.,* 1973), Moussiegt (1979, 1982, 1988), and Horsfall & Novak (1992). Recently, Briegel *et al.* (2001) studied physiological parameters of different *Ae. vexans* populations in the laboratory and from this study confirmed inter-population differences in body size and flight range.

Earlier attempts to colonise this species had limited success. Dashkina & Tsarichkova (1965) gave a brief report on cage matings of *Ae. vexans*, but did not mention further studies. Taylor & Brust (1974) stimulated some sexual activity in *Ae. vexans* by keeping adults in the same cage as a stenogamous strain of *Ae. dorsalis,* but failed to establish a self-reproducing colony. A strain from Champaign/ Urbana, Illinois, U.S.A. was maintained in the laboratory for more than 40 generations using induced copulation (i.e. forced mating) (McDaniel & Horsfall 1957; Horsfall & Taylor 1967). This paper describes the foundation and maintenance of laboratory colonies of *Ae. vexans* from many locations in Europe and North America. During this work it has become apparent that the strains colonised exhibit differences of adaptation to laboratory conditions, particularly with regard to stenogamy and this question is discussed later.

Insectory conditions

Environmental conditions in the insectory are maintained at 25±3^{0}C and 70±20% RH. The daily photoperiod of 17L: 7D is supplemented with automatically dimmed dawn and dusk phases of approximately 60 minutes each, using 40W fluorescent 'daylight' bulbs (4-8 in winter, 2-3 in summer), and 2 dimmed 75 W or 100 W plant grow light bulbs. The plant grow bulbs are at full intensity during the daylight phase and are dimmed to zero at dusk. Natural light conditions are necessary to maintain a high copulation rate over many generations, and a 72 cm x 77 cm window in the laboratory supplies natural light and fresh air. Moisture is provided by water in containers varying in size between 30 and 120 litres and aerated by 1-3 porous air stones. Water used for the rearing of larvae is maintained at 28°C by the use of aquarium heaters. This water is recycled and losses are replaced by rainwater from a hose connected to an outdoor container capable of holding 800 litres. Aquarium filter systems keep the water clean. Fans equipped with filters are used to reduce the establishment of mould cultures on the walls and floor, and to reduce allergens such as insect scales and dust.

The entire insectory, including the cages and dishes are thoroughly cleaned on a regular basis. Sponges used for feeding adults with honey are sterilised weekly in boiling water and cages and equipment are swabbed with ethyl alcohol as needed to control fungi. A heavy-duty vacuum cleaner and a heating fan (hair dryer) are used to eliminate spiders, silverfish, collembolans, ants and mites in the laboratory and in the cages. White insectory walls have proved helpful in detecting and eliminating escaped adult mosquitoes.

The gauze covered mosquito cages are of various designs and sizes including:

1. A 5 m^3 volume walk-in, double-entrance cage with netting sides and top, measuring 1 m x 2 m x 2.5 m high, and containing several potted 1-2 m high shrubs *(Hibiscus sinensis)* and bunches of grass *(Cyperus alternifolius)* to provide additional moisture, adult resting sites and swarming markers. The *Hibiscus* also provided a carbohydrate source; the leaves have large nectar glands near their base (Koernicke, 1918) where adults are observed feeding. The soil in the hibiscus pots is covered with fine stones and the grass pots are kept filled to the brim with water to prevent oviposition.
2. A 10 m^3 volume walk in, double entrance cage measuring 2 m x 2.5 m x 2 m, with the same shrub and grass furnishings.
3. A 1.4 m^3 volume walk in cage measuring 70 cm x 1 m x 2 m.
4. A 0.1 m^3 volume cage measuring 45 cm x 50 cm on the base, 43 cm x 48 cm on the top and 50 cm high The truncated pyramidal shape causes blood fed females resting on the netting sides to defecate on the PVC plastic cage bottom, facilitating cage cleaning.
5. An experimental design of oviposition cage of about 0.04 m^3 volume, measuring 35 cm x 25 cm x 50 cm, in which a 34 cm x 24 cm x 5 cm deep tray lined with an oviposition substrate was set at an angle of 25-30^0 from the horizontal by resting one end on a styrofoam block. This allowed the lower end of the substrate of mud mixed with leaf detritus to be immersed, and the remainder to provide a dampness gradient towards the dry upper end.
6. A standard oviposition cage of a design developed from the experimental oviposition cage. With a volume of roughly 0.03 m^3, this cage is 25 cm wide by 35 cm long and is higher (50 cm) at one end than the other (32 cm) to accommodate a floor angled at 30^0 from the horizontal into which a removable oval oviposition tray (29 cm long, 18 cm wide and 7 cm high) is tightly fitted. Instead of a mud/detritus mixture substrate, these oviposition trays have a 3-5 cm thick moss lining which, before being brought into use, had been flooded for several days in order to hatch and/or float off other organisms and then dried and stored until needed. The cosmopolitan moss *Calliergone cuspidata* (Loeske) is abundant locally, but sphagnum moss makes an equally good substrate. Water taken from pupal bowls is poured on to the moss until 1-2 cm depth of open water surface can be seen in the lowest portion of the tray. Water in which larvae and pupae have developed, if filtered after emergence and stored at 4°C is an oviposition attractant for *Ochlerotatus atropalpus* (Kalpage & Brust, 1973), *Oc. communis* (Maire & Langis, 1985), and *Aedes albopictus* (Allan & Kline, 1995) and is routinely used in this insectory.

Insectory procedures

A standardised insectory procedure has been established as described below.

Adults

Adult mosquitoes of established colonies are routinely kept in the truncated pyramidal cages. They are offered a blood meal every second or third day, either by the researcher placing the lower arm directly on top of the cage, or from a thermostatically controlled membrane feeder (Kuhn, 1998) filled with bovine blood being placed on top of the cage. Carbohydrates in the form of insecticide-free apple slices and honey-soaked plastic sponges, to which are added some crystals of isoleucine to enhance egg production (Chang & Judson 1979; Briegel 1985, 1986), are routinely placed on the top of the cages. The sponges are covered with plastic lids to reduce evaporation and drying. Cages taken from the insectory are covered with tight fitting plastic bags to maintain relative humidity at above 50%. In the early stages of colonisation, pupae emerge into a large 5 m^3 or 10 m^3 cage providing more space for swarming. They are also offered bloodmeals in this cage and some 6-10 days after emergence all females that have taken a blood meal are transferred to an oviposition cage.

Oviposition

The oval oviposition trays fit snugly into a hole cut into the angled white polyvinyl floor of the oviposition cage. Water taken from pupal bowls is poured on to the moss lining where it seeps downwards until 1-2 cm of open water surface can be seen in the lowest portion of the tray, creating a dampness gradient towards the upper end. As it is important during colony initiation to collect every fertile egg, oviposition trays are carefully fitted to be level with the cage floor in order to maximise chances of moribund gravid females reaching the oviposition substrate. The adults are left in the oviposition cage for varying periods, usually 3-4 weeks, after which the oviposition tray with its

substrate is removed, covered and stored at room temperature for at least a week to allow completion of embryonic development. The eggs are then ready to hatch and may be immersed or stored for longer periods as required.

Egg storage

Eggs may be stored at room temperature for several weeks. When held at 6±2^{0}C embryos enter a 'latent' or diapause phase (Wilson & Horsfall, 1970) and remain viable for several years if carefully monitored for moisture. However, for both short and long term storage, the lid of the oviposition dish should be perforated to allow for air exchange. In this laboratory a 5 mm hole is made at each end of the lid. Diapausing eggs are more or less frost resistant, and are best for shipping by airmail. To ensure continuing viability of long term refrigerator-stored eggs a small number of are reactivated each year.

Hatching

Embryonic diapause is terminated after holding eggs at 20-24^{0}C for 10-14 days (Wilson & Horsfall, 1970). After this conditioning period hatching is initiated by immersing the moss substrate containing eggs in a bucket filled to a depth of 10 cm with water at a temperature of 22-25^{0}C. Rainwater, or tap water which has been allowed to stand for several weeks to de-chlorinate, are both suitable. Approximately one litre of water containing fragments of moss, hay (Gjuillin *et al.*, 1941) or other plant material (Horsfall, 1956) is added, and the bucket is placed under a reflector lamp. The warmth of the lamp stimulates microbial activity in the plant material, and this decreases the level of dissolved oxygen in the water, a well-known method of inducting hatching of aedine mosquitoes (Gjuillin *et al.*, 1941). If properly conditioned, larvae begin to hatch 6-7 minutes after flooding. Occasionally a slight vacuum, known to aid hatching in *Aedes togoi* (Lee & Lee, 1994), is used to assist hatching when the eggs are first immersed. This is achieved by sealing one of the 5mm holes in the lid of the tray of moss substrate with elastoplast, and inserting into the other a fine glass pipette attached to a water tap suction unit.

Newly hatched larvae are transferred by hand or electrically operated pipettes to rearing basins using a modified electronic counter (Schulz, 1968) with an optical switch. This may be used to count larvae or pupae, but the most reliable counting results occur with late first instar larvae.

Rearing of larvae

Larval rearing is in white polyethylene basins (55 cm x 45 cm x 17 cm deep), each containing about 1000 larvae in 10-20 litres of rainwater or de-chlorinated tap water aerated with an air stone. The very young larvae feed on bacteria and other microbes in the moss and an added hay infusion (a nutritive broth of decaying organic material). Late first instar and larger larvae are fed on a slurry of the commercial fish food TETRAMIN® enriched with 10 % by weight of dried brewers yeast homogenised in water. For late first instar larvae, the food emulsion is screened through a commercial nylon filter, a metal coffee filter, or 25μm mesh plankton netting. The amount of food given is important and a small portion of this filtered emulsion is added twice daily to prevent accumulation of surplus food, which is detrimental, possibly due to mould development in the medium. The correct amount of food can be determined by checking the water twelve hours after feeding. Cloudy water indicates overfeeding and demands instant remedial action.

Pupae and adult emergence

Pupae are pipetted into containers containing clean water. If very large numbers are involved, the rearing water is poured through a stainless steel 1.1-1.2 mm mesh kitchen sieve that retains pupae but allows passage of any small larvae. To assist the survival of emerging adults, 1cm styrofoam flakes are scattered on the surface of the water. Pupae exhibit thigmotropism to floating objects and container walls and the floating flakes reduce alarm or escape movement, thus avoiding stress during metamorphosis. Moreover, emerging adults climb on to, and rest on the styrofoam flakes or the walls of the dish until the cuticle hardens and the wings become functional, thereby minimising mortality during metamorphosis.

Establishment and maintenance of cage colonies

Kühkopf (KK) strain

Material for the first *Ae. vexans* colony was obtained from the Kühkopf Nature Reserve (49 ^{0} 48' 58"N, 08 ^{0} 27' 56" E), a semi-artificial island in the River Rhine near Mainz, Germany in 1975. This being the first attempt at colonisation, procedures were largely experimental. More than 50000 third and fourth instar larvae were collected, of which approximately 8000 pupated. These were placed in the 5 m^3 cage, where the adults emerged and were given the opportunity of mating. Three to four days after emergence, the females were offered a human blood meal each day for 20-30 minutes. When 6-10 days old, all females having taken a bloodmeal were aspirated into the experimentally designed (0.04 m^3) oviposition cage containing a 34 cm x 24 cm x 5 cm deep tray with one end raised by a block to an angle of 25-30^0 from the horizontal. This tray contained an oviposition substrate consisting of a mixture of soil, grass, leaves and other organic debris from a natural *Ae. vexans* habitat. By adding water to this substrate, a moisture gradient was obtained in the tray from open water at the very bottom to only slightly moist soil and organic debris at the top of the slope. The oviposition tray was left in place until most of the females had died and then stored for 2 weeks at a temperature of 24-28^{0}C, after which the substrate was flooded to a depth of 10 cm. Addition of a moss infusion quickly reduced the dissolved oxygen (Gjullin *et al.*, 1939, 1941) and provided the stimulus for egg hatching. A total of 470 F_1 larvae were reared on a diet of ground up commercial fish food TETRAMIN®.

These procedures were repeated for the next 17 generations, each of which produced viable offspring. There was clearly a problem in the third laboratory generation but enough females mated to carry on the colony. After that stenogamy increased gradually, and by the eleventh generation it was possible to move the colony to a smaller (1.4 m^3) walk-in cage. By generation 22, stenogamy had increased to a level at which it was possible to maintain a strong colony in the 0.1 m^3 truncated pyramid cage. The KK strain is in the 158th cage generation and can be expanded at will. It is used as a stock colony well adapted to laboratory conditions and can now be reared in any insectory with natural light, day and night temperatures greater than 20^{0}C, and relative humidity greater than 50 %. Colony eggs, stored in moss, have been sent to several laboratories in Africa, Europe, Japan, and North America.

Blood fed females of the KK strain produce an average of 50-70 viable eggs, depending on individual body size. Though as many as 80% of a single hatch have survived to the adult stage, normally less than 50 % of hatching larvae do so. In the conditions of this insectary a generation takes 25-28 days in summer (Table 1) but, for unknown reasons, generation time is longer in winter. We therefore achieve a maximum of 10 generations per year.

Queich River (Q) strain

Material for this colony came from the River Queich near Offenbach on Queich in Southern Palatia, (49^0 12' 18" N, 08^0 02' 02" E) in 1989. The actual collection site is 6 km East of Landau in der Pfalz, and 13.5 km west of the Rhine.

This colony is the progeny of approximately 500 wild larvae taken to the laboratory and transferred to a small 0.1 m^3 volume truncated pyramid cage as they pupated. In comparison with the KK strain the Q strain proved much more stenogamous with a fertilisation rate of about 40 % in the females reared from field collected larvae. This colony is currently in the 22nd generation, and the size of the colony can be increased at will.

The habitat of this small population consisted of animal pastures and irrigated hayfields for possibly 600 years (Hassler *et al.*, 1995). With 3-5 irrigation floods for hay production per year, there were probably 3-5 generations of *Ae. vexans* annually. It is possible that this small population, having adapted to the regular irrigation pattern and the blood meal source provided by the pastured cattle, was isolated from other small, scattered, and more distant populations of the region, and that a long period of inbreeding increased natural stenogamy. The grazing livestock and most of the pastures have disappeared, but potential sources of human blood are available at the public swimming pool and the large sports centre that now occupy the region near the collecting site.

Minnesota (MN) strain

This colony is the progeny of material collected in the Rennville County (60 %) and the St Paul (40 %) regions of Minnesota.

In the summer of 1990 eggs were taken from a roadside ditch (44^0 46' 01" N, 94^0 52' 30" W) between the Chicago & Pacific railroad track and US Route 212 (Yellowstone trail), about 150kms west of St. Paul and near the Ballroom of Bird Island in Renville County, Minnesota This location was selected because as far as was known, no organised mosquito control with insecticides has been conducted in Renville County (Robert D. Sjogren, personal communication). Secondly, there are few lakes and creeks in the County, which optimised the finding of a geographically and/or behaviourally isolated stenogamous strain similar to the German Q strain.

During the same period biting females collected at Falcon Heights, St Paul and nearby Marine on the St Croix River were transferred to a standard 0.03 m^3 oviposition cage. Both eggs and cage were transported by air to Germany where the eggs from Renville County, and those laid by the St Paul area females in the cage were stored at 6 ± 2^0C for 6-8 months. From early March 1991, successive batches of these eggs were soaked at 10-14 days intervals. Eggs not hatching after the first immersion were dried and then soaked again, repeatedly if necessary, in order to capitalise on the delayed hatch response. The MN embryos appeared to display a response similar to that referred to by Wilson & Horsfall (1970) as 'instalment hatching'.

Approximately 15000 eggs between March and August 1991, and an additional 5000 eggs received from St Paul in September 1991, hatched and were reared in cohorts for the adults to emerge into the 10m^3 walk in cage, where a bloodmeal continued to be offered every 2-3 days. After a period of 10-14 days to allow for mating, all females having taken a bloodmeal were transferred to the oviposition cage containing the same oviposition substrate used by the parent generation. After oviposition had taken place the dish and substrate were placed in cold (6 ± 2^0C) storage for the winter months.

In February 1992, the oviposition substrate was removed from cold storage, conditioned (Horsfall, 1956) for 2 weeks at room temperature (22-24^0C) and submerged in hatching medium. Some 800 larvae hatched and were reared to produce 569 pupae to emerge in the 10 m^3 cage. 294 of this F_1 cage generation fed and were transferred to the oviposition cage where bloodmeals continued to be offered. When submerged in hatching medium several weeks later, this oviposition substrate produced a total of 419 F_2 females that took a bloodmeal. There was unusual larval mortality amongst the 1800 larval progeny of these females and only 373 bloodfed F_3 females were obtained. However, succeeding generations fared better with 1806 bloodfed F_4 females and 2879 bloodfed F_5 females. From our calculations, 4 -5 % of the F_5 females must have mated in the 1.4 m^3 cage and from the F_5 to the F_{13} generation, the MN colony was maintained in this cage. However, a parallel line was maintained in the large cage until the F_7 cage generation as a safety measure.

Repeated soakings were now producing plenty of larvae and several unsuccessful attempts were made between F_7 and the F_{11} generations to move the MN colony into the 0.1 m^3 cages used for maintaining stenogamous strains. Finally, by hanging two 0.1 m^3 cages inside the 10 m^3 cage a small F_{12} generation was obtained. The F_{13} generation was even smaller but thereafter the colony flourished in the 0.1 m^3 cage until a bottleneck occurred at generation F_{31}. The colony was returned to the larger 1.4 m^3 cage for one generation. Subsequent generations have all passed in the 0.1 m^3 cage. The colony is currently in generation 54, and can be increased at will. It can be maintained in any room with natural light where the temperature is above 20^0C and RH more than 50 %.

Winnipeg (WPG) strain

Eggs laid by *Ae. vexans* caught biting man within the city of Winnipeg, (49^0 53' 00" N, 97^0 09' 00" W). Manitoba Province, Canada in the summer of 1993, were transported by air to Germany, and processed by what were now standard methods. Approximately 4,000 larvae were reared to pupae to emerge in the 10 m^3 cage. A total of 609 blood fed females transferred to the oviposition cage did not produce sufficient viable eggs to start a self-perpetuating colony. Additional parent generation eggs were hatched and added to the few F_1 eggs already developing. However, as few individuals survived aquatic development and metamorphosis to at least take a partial bloodmeal, only 34 females of the F_1 cage generation were transferred to an oviposition cage. These produced 96 bloodfed F_2 females, which laid enough fertile eggs to continue with the fragile colony. A total of 111 bloodfed F_3 females were

succeeded by 441 bloodfed F_4 females. The next few cage generations continued to produce low numbers of viable females. When the time came to soak the eggs laid by the F_{11} generation, all unhatched eggs from previous generations were flooded again. Together these eggs yielded 1495 bloodfed F_{12} females, but the position of the colony was precarious.

Another supply of the eggs laid by field collected *Ae. vexans* from the same Winnipeg source was obtained in July, 1996. These were flooded simultaneously those of WPG strain F_{15} generation. Approximately 6000 larvae of wild parentage and 1200 F_{15} larvae were reared together and gave rise to 5089 blood fed females transferred from the 10 m^3 cage to the oviposition cage. The next 6 generations the colony were treated in the same way. At the same time a few females of each generation were moved to the 0.1 m^3 cage, and the eggs they laid were augmented by eggs laid by the 10 m^3 cage colony. However, by approximately the 26th generation, the 0.1 m^3 cage colony was self-sustaining and augmentation from the 10 m^3 cage colony was terminated. The small cage colony is stable and currently in the 42nd generation.

Portland (OR) strain

Material for this strain came from two sites along the banks of the Columbia River, near Portland, Oregon, USA in 1995. One site was 2.4 km upstream from Rooster Rock State Park, directly opposite Sand Island. The other site was a cattle pasture on Hayden Island, 8 km downstream from the Portland International Airport (45^0 37' 30" N, 122^0 42' 35" W). The eggs were transported to Germany, and stored for several weeks at 25^{0}C. When immersed, approximately 500 larvae from the Hayden Island field collection and 12 from the Rooster Rock site hatched. These larvae were reared and the adults emerged into the 10 m^3 walk-in cage. Ten days later 139 already blood fed females of this parent generation were transferred to the oviposition cage where they laid their eggs. After being stored for 10-14 days, these eggs were immersed in a moss infusion medium. Only about 300 larvae of this F_1 generation hatched, and parent generation eggs were therefore subjected to a second flooding. Only 7 hatched and these, combined with the F_1 larvae produced 64 females that fed on blood and went on to oviposit. These eggs produced approximately 1,000 larvae, of which 73 went on to become F_2 blood fed females. The succeeding F_3 and F_4 generations were more prolific, suggesting an increase in the proportion of stenogamous individuals in the 10 m^3 cage.

The eggs of the F_5 generation were stored at 6±2^{0}C for 6 months. Then, after 14 days of conditioning (Horsfall, 1956) at 24^{0}C, the eggs were flooded. Many more larvae hatched than in any of the previous generations and it was possible to transfer 4171 bloodfed females to oviposition cages. This colony was now stable enough to move to a the smaller 0.1 m^3 cage, where the F_6 generation mated so readily that approximately 600 females laid many thousands of fertile eggs. The colony is now routinely maintained in a 0.1 m^3 cage, and is currently in the 14th generation.

Colorado River (CAZ) strain

More than 1,000 biting females of *Ae. vexans* collected on the edge of the Laguna Dam of the Colorado River at a point (114^0 29' 40" W, 30^0 49' 30" N) on the California/Arizona border approximately 20 km northeast of Yuma, Arizona in March 1995, were placed in a portable oviposition cage, the bottom of which was completely filled by a tray containing moist soil and decaying plant debris from a muddy mosquito developmental site near the Laguna Dam. These females were blood fed daily and the cage containing both adults and eggs was transported by air to Germany.

On arrival in the laboratory, the oviposition tray was flooded and some 40000 larvae hatched. The adults emerged into the 10 m^3 cage, and were handled in a similar manner to the other strains. A total of 15655 females fed on blood and were transferred to standard oviposition cages. The F_1 eggs laid by these parental generation females were flooded in July 1995, when about. 5000 hatched. A high percentage the parent generation must have mated in the 10 m^3 cage, indicating that the population from which the CAZ strain was drawn contained a larger proportion of naturally stenogamous individuals than the populations from which the MN and WPG strains were drawn. In this respect the natural habitat of the CAZ population is largely isolated by the surrounding desert region. The MN and WPG strains were selected from natural populations known to have long dispersal ranges (Robert Sjogren, personal communication; Brust, 1980) contributing to a high rate of intermixture with neighbouring populations. Recently Briegel *et al.* (2001) evaluated the flight potential of different *Ae. vexans* strains in the laboratory and found maximum flights of females of 15-17 km per night

The F_1 larvae gave rise to 2,054 F_1 females that fed on blood and were transferred to the oviposition cage. When the F_2 eggs were flooded, a hatch of approximately 10000 larvae gave rise to 4025 F_2 females ready to contribute to the next generation.

At this point approximately 1500 F_2 pupae were transferred to a small 0.1 m^3 cage This attempt at small cage colonisation failed, but 16 F_3 larvae hatched, indicating that at least one F_2 female had mated in the small cage.

With regard to the main cage colony, only one third of the F_3 eggs were soaked for hatching and the rest were stored at a controlled temperature of $6^0 \pm 2^0$C. Nevertheless this colony was maintained in the 10 m^3 cage through three more generations without difficulty. It was then transferred to a small 0.1 m^3 cage where it is currently in its 23rd cage generation. Moreover, from the F_3 generation onwards it has been possible to place more eggs of each successive generation in cold storage than are necessary for colony maintenance.

In the spring of 2000 it was detected that CAZ is a mixed population with regard to diapause behaviour. When it was attempted to extract a thousand eggs (for shipping to a colleague) with a cold (4^0C) substrate, quite surprisingly, hundreds of larvae hatched. Since the seventh generation we have attempted to select for a non-diapause (CAZN) and diapause (CAZD) strain. Ambiguous results indicate that diapause is not under the control of a single gene.

Meteorological data from the Yuma, North Gila weather station confirm the working hypothesis that an *Ae. vexans* population from such a warm environment may have partially lost the ability to shift into diapause, which also means frost resistance. In contrast, frost resistance is obligatory for overwintering *Ae. vexans* eggs in Winnipeg / Manitoba and Renville County / Minnesota. Further studies of diapause in this field are currently under way.

Niagara River, Fort Erie (NIR strain)

Female *Ae. vexans* collected at College Road, about 1.2 km west of the Niagara Parkway, in the township of Fort Erie, Ontario Province (42^0 57' 15" N, 78^0 19' 20" W) in August 1996, laid eggs which were taken to the laboratory in Mainz, Germany. The first 7 cage generations of this strain were maintained in the 10 m^3 walk-in cage. However an additional colony in a small 0.1 m^3 cage was started at the 8th generation. By the 11th generation this small cage colony was stable and capable of being expanded. As with the CAZ strain, this strain was drawn from what may be a more or less isolated population.

Green River (GR) strain

Ae. vexans eggs from a known riverside developmental site of this species (40^0 18' 11" N, 109^0 21' 15", W) two km SE of the Interstate Highway 40 road bridge crossing Green River in northeastern Utah, were despatched to Mainz for colonisation in the spring of 1997. Using the standard procedures a cage colony of this strain was established and maintained for 4 generations in a 10 m^3 cage. Second cage generation material was also used to establish a branch colony in a small 0.1 m^3 cage. No difficulties were encountered and this 0.1 m^3 cage colony is currently in the 9th generation. It is completely autonomous and can be increased at will.

The high degree of natural stenogamy in this strain may possibly be due to geographical isolation of a local population, or a series of local populations, in the semi-arid area through which this river meanders and where seasonal shallows, backwaters and islands abound. Apart from the spring thaw, the Green River floodplain is susceptible to widespread flooding each time it is necessary to release water from the Flaming Gorge Dam - see *Vector Ecology Newsletter* (2000) **31** (2) page 7. However, much more research into this aspect is needed before question of a possible linkage of natural stenogamy and isolated Green Giver populations of *Ae. vexans* is resolved.

Ticino River (BOL) strain

Larvae and adults of *Ae. vexans* were collected in June 1998 from about 6 km from Locarno (46^0 09' 10" N, 08^0 52' 20" E) in a location in the Swiss "Bolle di Magadino" Nature Reserve, which includes the Ticino delta. Field-collected females were taken to the laboratory, put into an oviposition cage and offered a blood meal every 2-3 days. Pupae reared from the field collected larvae emerged into a 10 m^3 cage and females which fed on blood were transferred to the oviposition cage The substrate in the

oviposition basin was taken from the larval collection site, and was used both for oviposition by the field-collected females and those reared from field collected larvae. Because of small numbers, the oviposition substrate had to be alternately dried and reflooded and the parental and filial generations had to be amalgamated in the 10 m^3 cage. Eggs from these females were stored through the winter and immersed only in early 1999. More than 6,000 larvae hatched. This parental/filial mixture was referred to as the F_4 population, and was divided into a larger cohort to be maintained in the 10 m^3 cage, and a smaller cohort (approximately 1,500 individuals) to be maintained in the 0.1 m^3 cage. A month later, the oviposition substrate in the 0.1 m^3 cage was submerged in the moss infusion medium, and approximately 5000 F_5 larvae hatched. The colony has been maintained in the 0.1 m^3 cage ever since, and is currently in its 10th cage generation.

Discussion

Data are slowly being gathered to test the validity of the hypothesis, that some populations of the widespread species, *Ae. vexans,* because of geographical or other isolating mechanisms have deviated in certain aspects of behaviour. Populations inhabiting geographically or ecologically isolated situations with limited scope for dispersal and contact with other populations seem to be more or less stenogamous and this trait is quickly selected out in cage colonies, whilst, populations in areas without barriers to dispersal and intermixing with other populations have a lower potential for stenogamy and hence for colonisation.

Further information on this and other aspects of the population genetics of *Ae. vexans* throughout its range in the northern hemisphere is required. Researchers have reported different types and sizes of eggs (Horsfall *et al.*, 1973) and adults also differ in size and may exhibit behavioural differences as is evident in the strains we have colonised. Genetic compatibility can now be tested in the laboratory, by attempting biological crosses of strains from different geographical regions. Populations can now be analysed using electrophoresis, polymerase chain reaction (PCR) and DNA sequencing technology. Modern technology makes research into a host of subjects relevant to pest mosquito control possible, including subjects such as repellents, oviposition attractants, larvicide resistance management, disease vector competence (Halouzka, 1993, 1998; Hubalek *et al.*, 1998), and cytoplasmic incompatibility (Braig *et al.*, 1994). *Ae. vexans,* the most troublesome human and animal pest in the northern hemisphere over the past century, is an ideal subject for this research. The colonisation of the European and North American strains already achieved and the planned colonisation of further strains will facilitate research into these and other aspects of mosquito biology and control.

Acknowledgements

I am grateful for the warm hospitality extended by and/or generous help given in various ways in North America and Europe by Russ Boles, Ann and Reinhart Brust, Diane Crane, Alan Curtis, Peter De Chant, Bernd Klingenberg, Christel Kuhn, Timothy Kurtti, the late Professor Hannes Laven, Craig Levy, Peter Lüthy, Ulrike Munderloh, Jimmy Ohlsen, Susan Palchik, Clement Ramsdale, Nancy Read, Stephen Romney, Bob Sjogren, Chris Sumner and Jim Stark. The study was partly funded by the Metropolitan Mosquito Control District (MMCD).

References

Braig, H.R., Guzman, H., Tesh, R.B. & O'Neill, S.L. (1994) Replacement of the natural *Wolbachia* symbiont of *Drosophila simulans* with a mosquito counterpart. *Nature* **367**, 453-455.

Briegel, H. (1985) Mosquito reproduction: incomplete utilization of the blood meal protein for oögenesis. *Journal of Insect Physiology* **31**, 15-21.

Briegel, H. (1986) Protein catabolism and nitrogen partitioning during oögenesis in the mosquito *Aedes aegypti*. *Journal of Insect Physiology* **32**, 455-462.

Briegel, H., Waltert, A. & Kuhn, R. (2001) Reproductive physiology of *Aedes (Aedimorphus) vexans* (Diptera: Culicidae) in relation to flight potential. *Journal of medical Entomology* **38**, 557-565.

Brust, R.A. (1980) Dispersal behaviour of adult *Aedes sticticus* and *Aedes vexans* (Diptera: Culicidae) in Manitoba. *Canadian Entomologist* **112**, 31-42.

Chang, Y.Y.H. & Judson, C.L. (1979) Amino acid composition of human and guinea pig blood proteins, and ovarian proteins of the yellow fever mosquito *Aedes aegypti*, and their effects on the mosquito egg production. *Comparative Biochemistry and Physiology* **62A**, 753-755.

Dashkina, N.G. & Tsarichkova, D.B. (1965) Copulation of some mosquito species of the genus *Aedes* in the laboratory. [in Russian with an English abstract] *Meditsinskaya Parazitologiya i Parazitarnye Bolezni* **34**, 235

Gjuillin, C.M., Yates, W.W. & Stage, H.H. (1939) The effect of certain chemicals on the hatching of mosquito eggs. *Science* **89**, 539-540.

Gjuillin, C.M., Hegarty, C.P. & Bollen, W.P. (1941) The necessity of low oxygen concentration for the hatching of *Aedes* mosquito eggs. *Journal of Cellular and Comparative Physiology* **17**, 193-202.

Halouzka, J. (1993) Borreliae in *Aedes vexans* and hibernating *Culex pipiens molestus* mosquitoes. *Biologia (Bratislava)* **48**, 123-124.

Halouzka, J., Postic, D. & Hubalek, Z. (1998) Isolation of the spirochaete *Borrelia afzelii* from the mosquito *Aedes vexans* in the Czech Republic. *Medical and Veterinary Entomology* **12**, 103-105.

Hassler, D., Hassler, M. & Glaser, K.-H. (1995) *Wässerwiesen - Geschichte, Technik und Ökologie der bewässerten Wiesen, Bäche und Gräben in Kraichgau, Hardt und Bruhrain. Beihefte zu den Veröffentlichungen. für Naturschutz und Landschaftspflege in Baden-Württemberg (Karlsruhe)* **87**. 432 pp

Horsfall, W.R. (1955) *Mosquitoes. Their bionomics and relation to disease.* Ronald Press. New York, U.S.A. 723 pp.

Horsfall, W.R. (1956) Eggs of floodwater mosquitoes III (Diptera: Culicidae). Conditioning and hatching of *Aedes vexans*. *Annals of the Entomological Society of America* **49**, 66-71.

Horsfall, W.R., Fowler, Jr. H.W., Moretti, L.J. & Larsen, J.R (1973) *Bionomics and embryology of the inland floodwater mosquito Aedes vexans.* University of Illinois Press, Urbana, IL, 211 pp.

Horsfall, W.R. & Novak, R.J. (1991/92) The inland floodwater mosquito *Aedes vexans*: an annotated bibliography to world literature, part I / part II (on HD-diskettes), AMCA Central Office, 2200 E. Prien Lake Road, Lake Charles, LA 70601, USA.

Horsfall, W.R. & Taylor, M.L. (1967) Temperature and age as factors in inducing insemination of mosquitoes (Diptera: Culicidae). *Annals of the Entomological Society of America* **60**, 118-120.

Hubalek, Z., J. Halouzka, & Z. Juricova. (1998) Investigation of haematophagous arthropods for *Borrelia* - summarized data, 1988-1996. *Folia Parasitologica* **45**, 67-72.

Kalpage, K.S.P. & Brust, R.A. (1973) Oviposition attractant produced by immature *Aedes atropalpus* (Diptera: Culicidae). *Environmental Entomology* **2**, 729-730.

Koernicke, M. (1918) *Über die extrafloralen Nectarien auf den Laubblättern einiger Hibisceen. Flora* **111/112**, 526-540 + Pl VII.

Kuhn, R. (1998) An affordable and handy "Membrane Mouse" ("MM"). [Poster Abstract P-6] *Acta Parasitológica Portuguesa* **5**, 15.

Lee, D. K. & Lee, J.S. (1994) Effect of low air pressure on hatching of *Aedes togoi* (Culicidae, Diptera) eggs. *Korean Journal of Entomology* **24**, 57-62.

Maire, A. & Langis, R (1985) Oviposition responses of *Aedes (Ochlerotatus) communis* (Diptera, Culicidae) to larval holding water. *Journal of Medical Entomology* **22**, 111-112.

McDaniel, I.N. & Horsfall, W.R. (1957) Induced copulation of aedine mosquitoes. *Science* **125**, 745.

Moussiegt, O. (1979) *Aedes (Aedimorphus) vexans* (Meigen, 1830) Bibliographie. Document Entente Interdépartementale pur la Démoustication du Littoral Méditerranéen Françcais, Montpellier (France), n° **42**, 57 pp.

Moussiegt, O. (1982) *Aedes (Aedimorphus) vexans* (Meigen, 1830). Bibliographie (Supplement). Document Entente Interdépartementale pur la Démoustication du Littoral Méditerranéen Français, Montpellier (France), n° **46**, 94 pp.

Moussiegt, O. (1988) *Aedes (Aedimorphus) vexans* (Meigen, 1830). Bibliographie - 2e supplément, Document Entente Interdépartementale pur la Démoustication du Littoral Méditerranéen Français, Montpellier (France), n° **56**, 57 pp.

Nelson, R.L. (1971) Mosquito studies (Diptera: Culicidae) XXVI. Winter biology of *Culex tarsalis* in Imperial Valley, California. *Contributions of the American Entomological Institute* **7**, 1-56.

O'Malley, C.M. (1990) *Aedes vexans* (Meigen): An old foe. *Proceedings of the New Jersey Mosquito Control Association*, 90-95.

Schulz, H. (1968) Ein einfaches Gerät zum Zählen von Fischeiern. *Berichte der Deutschen Wissenschaftlichen Kommission für Meeresforschung* **19**, 226-230.

Taylor, B.W. & Brust, R.A. (1974) Laboratory mating of *Aedes vexans* (Diptera: Culicidae). *Annals of the Entomological Society of America* **67**, 137-138.

Wilson, G.R. & Horsfall, W.R. (1970) Eggs of floodwater mosquitoes XII. Hatching of *Aedes vexans* (Diptera: Culicidae). *Annals of the Entomological Society of America* **63**, 1644-1647.

Table 1

Rearing Schedule

Insectory with a temperature of 25 ± 3° C and RH 70 ± 20%

1st week	2nd	3rd	4th	1st/5th
M T W T F S S	M T W T F S S	M T W T F S S	M T W T F S S	M ...
8 a.m. flooding of eggs, remove scum	S M first pupae → cages with moist oviposition substrate			**8 a.m.** flooding of eggs...
	T W first emerging adults			
	keep the oviposition substrate moist → → → → **F** let the substrate dry			
feeding of larvae |				
	honey-sponges and apple slices on top of the cages for ♀♀ and ♂♂			
aeration of holding water				
	T last pupae, discard the rest.			
	F change water.	**F S** first blood meal → → → → (if a generation period of 4 weeks is intended)		
		mating and host-seeking behaviour		
5 p.m.: feeding of larvae |		egg formation		first larvae
		oviposition		will be ready
		embryonic development →		to hatch

In the beginning and during the winter a rearing period of 5 or even 6 weeks would be better.