AUTOGENY AND REARING OF *CULICOIDES FURENS*, *C. HOLLENSIS* AND *C. MELLEUS* (DIPTERA: CERATOPOGONIDAE) FROM COASTAL NORTH CAROLINA[1]

H. G. KOCH[2] AND R. C. AXTELL

Dept of Entomology, N. C. State University, Raleigh, N. C. 27607

ABSTRACT. Autogeny was demonstrated by newly emerged adults of *Culicoides furens* (Poey), *C. hollensis* (Melander & Brues) and *C. melleus* (Coquillett) collected from coastal salt marsh and tidal creek areas of North Carolina. This is the first demonstration of autogeny in a population of *C. hollensis*. The mean number of eggs laid on agar or moist filter paper by adults (fed only on a 10% sucrose solution) held individually at 20–22°C and 85–90% RH under natural lighting were: *C. furens* 39.9 (± 23.5), *C. hollensis* 54.0; (± 38.2) and *C. melleus* 54.5 (± 21.8). Egg hatch was 90.4% for *C. melleus*, 77.7% for *C. hollensis* and 45.5% for *C. furens*. About 15% of the *C. furens*, 13% of the *C. hollensis* and 0.3% of the *C. melleus* were reared to the adult stage from eggs laid autogenously on agar medium supplied with live nematodes as food for the larval stages.

Autogeny has been established in many species of *Culicoides* (Kettle 1977, Lindley 1966). *C. furens* (Poey) and *C. melleus* (Coquillett) were found to mature the first batch of eggs without benefit of a bloodmeal (Linley 1966, Linley & Hinds 1976), but no information is available on *C. hollensis* (Melander & Brues). Most information on autogeny has stressed egg development in ovarioles and not the actual oviposition and viability.

Preliminary success (Koch and Axtell 1977) in rearing late instar field-collected larvae of *C. furens*, *C. hollensis* and *C. melleus* to the adult stage using a new simple technique (Kettle et al. 1975) suggested the possibility for rearing complete generations by this method and eventual colonization. Older methods of colonization for *C. furens* (Linley 1968, 1969) and *C. melleus*[3] are very tedious and time consuming.

Studies were conducted with *C. furens*, *C. hollensis* and *C. melleus* to determine (1) the proportion of field collected adult females without a blood meal that would oviposit on agar substrate, (2) egg viability (and hence the degree of autogeny), and (3) success in rearing the newly hatched larvae to the adult stage by adding living nematodes to the agar as food.

MATERIALS AND METHODS

ADULT COLLECTING AND HANDLING. Areas known to contain large populations of larvae (Kline and Axtell 1975, 1977) were selected in 1976 to collect newly emerged adult *C. furens*, *C. hollensis* and *C. melleus* by means of galvanized metal conical emergence traps set over the soil. The newly emerged gnats moved upwards from the soil and were captured in glass jars lined with moist filter paper to prevent damage to the specimens from condensation. Traps were placed in a salt marsh having *Spartina alterniflora* Loiseleur as the dominant vegetation and located at the end of Lake Shore Drive, along the Newport River, Morehead City, North Carolina. Fifteen traps were in short (<0.3m) *S. alterniflora* for collecting primarily *C. furens* and 15 were along a

[1] This research was supported by NOAA, Office of Sea Grant, U. S. Department of Commerce, under Grant No. 04-3-158-40 and the North Carolina Department of Administration. Paper No. 5475 of the Journal Series of the North Carolina Agricultural Experiment Station.

[2] Present address: Lone Star Tick Laboratory, USDA, ARS, P. O. Box 588, Poteau, OK 74953.

[3] Linley, J. R. 1968. *Culicoides melleus* as a laboratory animal. Ceratopogonidae Information Exchange No. 1:5–6.

major drainage ditch in tall (>1.2m) *S. alterniflora* for collecting primarily *C. hollensis*. A sandy intertidal zone at nearby Atlantic Beach, North Carolina was used for collecting *C. melleus* by 10 traps along a non-vegetated margin of a tidal creek.

Collections were retrieved in the morning following 12–24 hours of trapping and each species was identified and released into separate mating cages (Linley 1968). Each cage (66 x 38 x 51 cm high) had a 20 cm square Plexiglas® sliding door in the front of the cage, the top, sides and back covered with fine mesh white organdy cloth, and the bottom made of white-painted masonite. Food was supplied by vials containing 10% sucrose solution with protruding cotton wicks and suspended inside the cages. Cages were held indoors at 20–22°C near an east-facing window to provide natural lighting. The insects were held at least 48 hr before use in the experiments.

OVIPOSITION CONTAINERS. Commercial agar was dissolved in near-boiling water (1 g agar/100 ml water) (Kettle et al. 1975). About 15 ml of the hot agar solution was poured into plastic vials (9.5 cm x 3 cm diam), allowed to cool, the insides of the vial wiped with tissue to remove condensation, and a disc of filter paper (2.5 cm diam) was placed in the center of the agar surface. The disc served as a resting and oviposition site for the insects and, additionally, aided in controlling surface evaporation. A small hole (2.2 cm diam) was punched in the center of the plastic lid of each vial and a 6 cm square of white organdy cloth positioned over the vial top before snapping the lid in place to provide for air exchange and a resting surface for the *Culicoides*.

Individual adult females of the 3 species were aspirated from the mating cages and transferred to the oviposition containers. A small cotton ball (<0.5 cm diam) was soaked in 10% sucrose solution and placed on the vial cloth top for food. Vials of each species were placed into a "10-gallon" aquarium (51.5 x 26.5 x 32 cm high) with glass top providing 85–90% RH. Aquaria were placed in the insectary near the mating cages under similar temperature and lighting conditions. Each vial was examined for egg deposition and egg hatch at least once every 24 hrs. until the females died.

REARING CONTAINERS. Small plastic petri dishes (3.5 cm diam x 1.0 cm high) and large circular plastic dishes (15.2 cm diam x 4.0 cm high) were prepared by adding 3 ml and 300 ml, respectively, of the hot agar media. The dishes were placed on a 15–20° incline and the medium allowed to gel. The final slanting agar prevented excess water from completely covering the surface and slowing air exchange. The lids of the large dishes had a 2-cm diam. opening covered with 200-mesh bronze screen to allow air exchange. In addition, screw-top clear plastic vials (4.5 cm diam x 8.8 cm high) were used with each containing 40 ml of agar media allowed to set on a slight angle. The lids were left partially unscrewed to allow air exchange.

LARVAL FOOD AND REARING PROCEDURES. The *Culicoides* larvae were provided with nematodes, *Panagrellus redivivus* (L.)[4] which were cultured in the laboratory on oatmeal and yeast. Nematodes were swabbed with a small paintbrush from the sides of the plastic culture boxes (30.5 x 16.5 x 8.8 cm high) and suspended in a small quantity of water prior to adding 3–5 ml every 3–4 days to the *Culicoides* rearing containers.

All of the various types of rearing containers were held inside the covered aquaria and examined every 3–4 days for the presence of the larvae, pupae and adult stages. A single egg of either *C. furens* or *C. melleus* was transferred from an oviposition container to the agar surface in each small rearing container. Groups of *C. hollensis* and *C. melleus* eggs (91–342) were held in the large rearing containers. Several groups of first instar *C. furens* and

[4] Obtained from Aqua Engineers, 250 Cedar St., Ortonville, Michigan 48462, USA and identified by Dr. H. H. Triantaphyllou, Dept. of Plant Pathology, North Carolina State University.

C. melleus larvae which hatched from eggs laid in the oviposition vials were transferred to the screw-top rearing containers. This was done by removing a cylinder of agar containing the larvae from an oviposition vial and placing it on the agar surface in a larger rearing vial. The agar cylinders served as added larval substrates and as pupation sites above any water accumulation resulting from adding the nematode suspension. Vials were held and observed along with the other rearing containers.

In addition, the success of rearing from groups of eggs oviposited directly on the agar in the screw-top containers was determined. This was done in 27 vials with 10–17 adult female *C. furens* from the mating cage added to each vial for 2 days after which the eggs were counted and the organdy cloth top replaced with the loose vial cap. These vials received nematodes and were held and observed in the same manner as the other rearing containers.

RESULTS

The agar surface provided a suitable substrate for oviposition by the female *Culicoides*. Most of the eggs were laid on the agar surface adjacent to the side of the vial. A few females oviposited on the filter paper. Any small depressions in the agar, such as those resulting from trapped air bubbles after pouring the hot medium, were used for oviposition.

Most eggs were laid within 1–2 days after the adults were transferred to the oviposition containers. Some *C. furens* and *C. hollensis* females did not lay eggs for 1–2 weeks, and most of these eggs from delayed depositions were not viable. *C. melleus* females generally laid eggs sooner than the other species.

Comparative results for the 3 species are summarized in Table 1. The portion of the adult female *Culicoides* autogenously laying eggs differed among the species. About 65% of the 144 *C. melleus*, 52% of the 90 *C. hollensis* and 26% of the 254 *C. furens* laid eggs. Females which died before the 1st observation period were not considered in the percentage calculations. The distribution of the number of eggs laid by a female *Culicoides* differed among the species. A normal distribution occurred for *C. melleus* with most (79%) of the females laying 41–80 eggs each. All of the *C. furens* and most (68%) of the *C. hollensis* laid 80 or less eggs each. A comparatively large number (32%) of the *C. hollensis* and 7% of the *C. melleus* females laid more than 80 eggs. The average number of eggs produced per laying female and the percent hatch was variable for each species. *C. furens* females averaged only 39.5 eggs each with 45.5% hatching, *C. hollensis* females averaged 54.0 eggs each with 77.7% hatching, while

Table 1. Number of recently emerged field-collected autogenous female *Culicoides* laying eggs in ovipositional containers, average egg production and percent hatch.

	C. furens	*C. hollensis*	*C. melleus*
No. females held	254	90	144
No. females ovipositing	66	47	93
No. females laying eggs within range:			
1–20	22	15	9
21–40	12	4	9
41–60	20	7	40
61–80	12	6	28
81–100	0	7	6
101–120	1	3	1
Mean (± SD) no. eggs per ovipositing female	29.9 ± 23.5	54.0 ± 38.3	54.5 ± 21.8
Percent hatch	45.5	77.7	90.4

C. melleus females produced an average of 54.5 eggs each with most of them hatching (90.4%). The mean number of viable eggs produced per female collected was greatest for *C. melleus* (31.8 ± 12.7) followed by *C. hollensis* (21.9 ± 15.5) and *C. furens* (4.24 ± 2.8).

The production of adult *Culicoides* from the eggs of autogenous adults in the different rearing containers was low although most adults appeared normal and readily flew in the small rearing containers. About 12% of the 210 individual and 15% of the 5596 grouped *C. furens* larvae reached the adult stage. There was little difference in production between the groups with or without the agar cylinders. About 13% of the 350 *C. hollensis* larvae completed development to the adult stage in the large plastic dishes. Very few of the *C. melleus* larvae (0.3%) in any of the rearing containers reached the adult stage.

DISCUSSION

This is the first report of a population of *C. hollensis* exhibiting autogeny but this phenomenon has been well documented in *C. furens* of Jamaica and Florida (Linley 1966, Linley et al. 1970) and *C. melleus* of Florida (Linley and Hinds 1976) and South Carolina (Henry 1973). Although Henry (1973), working in South Carolina, was unable to show autogeny in *C. hollensis*, only a few individuals from emergence traps were examined for egg maturation. The number of eggs oviposited on filter paper from blood-fed adults was reported ranging from 8 to 59 by this worker and up to 30 by Atchley and Hull (1936) (as *C. canithorax*). This is considerably less than the numbers of eggs we found deposited by *C. hollensis* without a blood meal. For *C. furens* and *C. melleus* the number of eggs we found was in the same range as those reported from other areas (Linley 1976).

The rather large number of adult females failing to lay eggs was probably due to the lack of mating (Linley, J. R., personal communication). This was particularly prevalent in *C. furens* and was further evidenced by the comparatively low viability of the eggs that were laid. *C. melleus* which is known to readily mate in confined spaces (Linley and Adams 1972a) oviposited viable eggs nearly twice as well with *C. hollensis* somewhat intermediate. Swarming is probably necessary in *C. furens* (Linley 1968) and possibly *C. hollensis*. Increasing the mating cage depth by at least 2-fold might allow sufficient space for the pairing of species requiring swarms and subsequently result in larger numbers of viable eggs.

Although *C. melleus* produced the greatest number of viable eggs per female collected, most of these could not be reared to the adult stage. Modifications of the diet or substrate will have to be made if large numbers of adults of this species are to be produced. This species is a predator of protozoa, motile algae and small invertebrates (Linley 1976) that are found in abundance in Florida *Culicoides* breeding areas (Linley and Adams 1972b).

The rearing of *C. furens* on the agar media was fairly successful and the time from egg to adult was similar to that reported by Linley (1969) using natural sterile substrate and a different species of nematode. *C. hollensis* is apparently equally adaptable to this rearing procedure. Cannibalism was apparently not a problem in rearing either *C. furens* or *C. melleus* since similar percentages of grouped or individual larvae emerged to the adult stage. *C. hollensis* was not reared individually.

Although living nematodes were the only food intentionally supplied to the *Culicoides* larvae in the agar substrate, the media was not sterilized and various microorganisms were probably present on the agar during the somewhat lengthy (1–3 months) rearing procedure. The addition of the nematodes every 3–4 days after swabbing them from the side of the culture containers also provided access for various microorganisms.

This rearing procedure has many advantages as outlined by Kettle et al. (1975) which include a transparent substrate for

unhindered observations of the life stages and no need for adding antibiotics or nutrients. In addition, *Culicoides* naturally oviposit on the agar surface and various organisms and nutrients can be added as larval food. By using sterilized medium, vials and eggs this procedure could be used to study the nutritional requirements of *Culicoides* larvae.

Literature Cited

Atchley, F. O. and J. B. Hull. 1936. Oviposition by *Culicoides* breeding in salt marshes. J. Parasitol. 2:514.

Henry, L. G. 1973. Biting midges (*Culicoides* spp.) of coastal South Carolina. Ph.D. Thesis, Clemson Univ., Clemson, South Carolina. University Microfilms, Ann Arbor, Michigan.

Kettle, D. S. 1977. Biology and bionomics of bloodsucking ceratopogonids. Annu. Rev. Entomol. 2:33–51.

Kettle, D. S., C. H. Wild and M. M. Elson. 1975. A new technique for rearing individual *Culicoides* larvae (Diptera: Ceratopogonidae). J. Med. Entomol. 12:263–64.

Kline, D. L. and R. C. Axtell. 1975. *Culicoides melleus* (Coq.) (Diptera: Ceratopogonidae): Seasonal abundance and emergence from sandy intertidal habitats. Mosquito News 35:328–34.

Kline, D. L. and R. C. Axtell. 1977. Distribution of *Culicoides hollensis, C. furens,* and *C. bermudensis* in relation to plant cover in a North Carolina salt marsh (Diptera: Ceratopogonidae). J. Med. Entomol. 13:545–52.

Koch, H. G. and R. C. Axtell. 1977. Agar rearing of *Culicoides. In:* World Ceratopogonidae Group, Proceedings of Meetings, Aug. 23–Sept. 1, 1976. Mosquito News 37:285.

Linley, J. R. 1966. The ovarian cycle in *Culicoides barbosai* Wirth and Blanton and *C. furens* (Poey) (Diptera: Ceratopogonidae). Bull. Entomol. Res. 57:1–17.

Linley, J. R. 1968. Colonization of *Culicoides furens.* Ann. Entomol. Soc. Amer. 61:1486–90.

Linley, J. R. 1969. Studies on larval development in *Culicoides furens* (Poey) (Diptera: Ceratopogonidae). I. Establishment of standard rearing technique. Ann. Entomol. Soc. Amer. 62:702–11.

Linley, J. R. 1976. Biting midges of mangrove swamps and saltmarshes (Diptera: Ceratopogonidae), pp. 335–76. *In* Marine Insects, L. Cheng, (ed.). American Elsevier, New York. 581 pp.

Linley, J. R. and G. M. Adams. 1972a. A study of the mating behavior of *Culicoides melleus* (Coquillett) Diptera: Ceratopogonidae). Trans. R. Entomol. Soc. Lond. 124:81–121.

Linley, J. R. and G. M. Adams. 1972b. Ecology and behavior of immature *Culicoides melleus* (Coq.) (Diptera: Ceratopogonidae). Bull. Entomol. Res. 62:113–28.

Linley, J. R. and M. J. Hinds. 1976. Seasonal change in size, female fecundity and male potency of *Culicoides melleus* (Diptera: Ceratopogonidae). J. Med. Entomol. 13:151–56.

Linley, J. R., F. D. S. Evans and H. T. Evans. 1970. Seasonal emergence of *Culicoides furens* (Diptera: Ceratopogonidae) at Vero Beach, Florida. Ann. Entomol. Soc. Amer. 63:1332–39.

Review of Applied Entomology, Series B.

The papers published in MOSQUITO NEWS are selectively abstracted and indexed in the REVIEW OF APPLIED ENTOMOLOGY, compiled by the Commonwealth Institute of Entomology, London and published by the Commonwealth Agricultural Bureaux. The REVIEW OF APPLIED ENTOMOLOGY, Series B (Medical and Veterinary) is published monthly and contains approximately four times as many abstracts on mosquitoes as TROPICAL DISEASES BULLETIN. There were 975 mosquito items in 1977. MOSQUITO NEWS is glad to correct an impression given in our December 1977 number concerning these two excellent publications.