

# TEMPORARY SMALL-SCALE COLONIZATION OF THE BEFFA FORM OF THE *SIMULIUM DAMNOSUM* THEOBALD COMPLEX (DIPTERA: SIMULIIDAE) IN AFRICA

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**ABSTRACT.** Two populations of the Beffa form of *Simulium damnosum* s.l. were colonized on a small scale for the first time in Africa. The colonies survived for three and five generations, respectively. Information gained led to improvements in adult handling techniques which proved adequate for colony maintenance despite the small numbers reared. Nevertheless, large scale rearings are required to avoid inbreeding and to produce sufficient numbers for experimental purposes.

## INTRODUCTION

Much recent progress has been made towards the laboratory colonization of members of the *Simulium damnosum* Theobald complex, the major vectors of *Onchocerca volvulus* (Leuckart) in Africa. Because most investigators have been supported, or supplied with eggs, by the WHO Onchocerciasis Control Programme, they have worked with West African onchocerciasis vectors. Cupp et al. (1981) reported complete generation rearing, with production of F<sub>1</sub> adults, of *S. damnosum* s.l. from the Volta River (Senchi Rapids) in southeastern Ghana. This was achieved in spite of an insemination rate of <1%. Simmons and Edman (1982) established the first multiple generation colony of *S. damnosum* s.l. with material from the same source. Their colony material was identified morphologically by Dr. R. Garms as "*Simulium soubrensel sanctipauli* heterozygotes." Soon afterwards Simmons et al. (1983) cytologically identified the West African laboratory mating form as "Beffa," a form of *S. soubrense/S. sanctipauli* (Meredith et al. 1983).

Simmons and Edman (1982) reported a shift in the male:female ratio of their colony from 1:1 in the F<sub>1</sub> to 0.07:1 in the F<sub>4</sub> generation. They also recorded that survival from first instar larva to adult dropped from 74 and 85% for the F<sub>1</sub> and F<sub>2</sub> generations respectively, to 18% in the F<sub>4</sub> and 0 in the F<sub>5</sub>. They suggested disease, algal blooms and inbreeding depression, resulting from the small number (3) of females that started the colony, as possible causes of these anomalies. Simmons et al. (1983) emphasized the need for recolonization of the Beffa form to study these potentially serious

obstacles to permanent colonization. They reared Beffa for one generation in Ghana and found that populations of this form from the Mono and Okpara rivers (bordering Togo/Benin and Benin/Nigeria respectively) had a high laboratory mating rate. This raised hopes that long-term colonization of these populations was feasible.

Our paper describes attempts to recolonize the Beffa form at Akosombo, Ghana, using simple apparatus and techniques.

## MATERIAL AND METHODS

Material was obtained from two different sites: Kaboua (08°14'N, 02°44'E) on the Okpara River and the Bégba Rapids (06°55'N, 01°35'E) in the Mono River, just south of Tohoum.

Many of the techniques employed were similar in principle to those of Simmons et al. (1983) but differed in detail.

**COLLECTION AND TRANSPORTATION OF FIELD MATERIAL.** Pupae from the Bégba Rapids were collected from vegetation in the river and placed in plastic bags. Kaboua females were captured coming to man but not allowed to feed. All material was kept cool during transportation to Akosombo.

**MAINTENANCE OF ADULTS.** In the laboratory, pupae were placed in a cage in which the emerged adults were attracted by light into a collecting device. Each fly was then tubed separately, provided with sugar solution and maintained at moderate temperatures and high humidity (Raybould et al. 1982).

**MATING.** The mouth aspirator method of Simmons and Edman (1978, 1982) was employed but a smaller tube of about 30 × 0.6 cm. was used. So that each coupling could be observed and the mated females separated, only about three flies of each sex were taken up together.

**BLOOD FEEDING.** Laboratory emerged and wild caught females were fed on man in the laboratory by holding the open-end of a 4.5 × 1.5 cm plastic tube containing a single fly against the skin of the lower arm. The fly was pe-

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riodically knocked down onto the skin by tapping the tube.

**OVIPOSITION INDUCEMENT.** Gravid females were induced to oviposit using the Simmons and Edman (1982) "twilight method" but as described by Boakye and Raybould (1985). Pieces of netting or corks on which eggs were laid were placed in a container of well aerated water until the next day when developing and undeveloped eggs were counted.

**REARING LARVAE AND COLLECTION OF ADULTS.** Developing egg batches were put in a gravity trough rearing system and the larvae fed on living algae and Tetra® fish food (Raybould et al. 1982). The original apparatus was modified by the replacement of the adult collecting device by a sheet of nylon netting covering the entire rearing system. Emerged adults were collected from under the net with an aspirator. Some late instar larvae were fixed by Carnoy's solution for cytological determination.

## RESULTS

Two colonies of the Beffa form were established, from Kaboua and the Bégba Rapids. Both colonies were started with only one female and allowed to remain small to observe for possible effects of inbreeding.

**KABOUA COLONY.** This colony was started with a single female and maintained for more than five generations by rearing one egg batch only per generation (Tables 1 and 3). The original parent female was collected at Kaboua on September 18, 1981 and blood-fed and induced to oviposit in the laboratory. The F<sub>1</sub> larvae developed well until a long power failure killed those that had not yet pupated. The pupae survived and 202 adults were obtained of which 138 were males and 60 were females (4 escaped prior to identification). Males were predominant because female larvae develop more slowly and many died during the power failure. In later generations, larval mortalities were high. However, successful mating and blood-feeding

and good female survival enabled the retention of the colony in spite of the paucity of adults (Table 1).

The mating rate of F<sub>1</sub> adults was high but only nine females blood-fed. One batch of fertile F<sub>2</sub> eggs was reared. The F<sub>2</sub> adults, 22 females, 10 males and 3 escapes, showed little mating activity but 2 females mated (probably with the same male). One fertile egg batch was reared to F<sub>3</sub> adults and 12 females and 10 males emerged. This time the males mated vigorously, but again the females were difficult to feed. One fertile egg batch was obtained. Only 9 F<sub>4</sub> adults (6 females and 3 males) were reared and three of the females emerged after all the males had died. Nevertheless, a fertile egg batch was laid and 43 F<sub>5</sub> adults were reared.

More F<sub>3</sub> females mated, blood-fed and survived to lay eggs than did those of earlier generations (Table 3). Six egg batches were obtained but only two were partly fertile with 12 and 40 developing eggs respectively. Only 10 eggs from one batch hatched although others developed to the advanced "eye-spot stage." The colony ended when the 10 larvae died at an early stage.

There was no consistent shift in the sex ratio during the life of the colony and Dr. R. A. Cheke, who examined the reared adults, found no morphological changes apart from one gynandromorph in the F<sub>3</sub>. The adults were used as voucher specimens when the Beffa form was described (Meredith et al. 1983). Some F<sub>1</sub> and F<sub>5</sub> larvae were studied cytotaxonically by Dr. S. E. O. Meredith and once again no changes were apparent in the few larvae examined.

**BÉGBA RAPIDS COLONY.** Several attempts were made to colonize the Mono River Beffa population at Bégba Rapids. In most cases sufficient F<sub>1</sub> adults were reared and some of the females mated and took blood but most of them died before ovipositing. One colony was established and maintained for three generations (Table 2).

The colony was started with a single female

Table 1. Life history of the Kaboua colony, Beffa form of *Simulium damnosum* s.l.

Generation	Total adults (male:female)	No. of mated females	No. fed (refed)	Egg batches Fertile/Total	Egg batches reared	Fertile eggs in reared batch	% survival to adults
Wild caught							
♀ parent	1	—	1	1/1	1	426	
F <sub>1</sub>	202(2.3:1)	Many	9(1)	2/3	1	211	47.4
F <sub>2</sub>	35(0.5:1)	2	3	2/2	1	250	16.6
F <sub>3</sub>	22(1.2:1)	5	3(1)	1/1	1	Not counted	8.8
F <sub>4</sub>	9(0.5:1)	1	1(1)	1/1	1	369	?
F <sub>5</sub>	43(0.6:1)*	13	17(1)	2/6	—	—	11.7

\* 14 late instar larvae removed and identified cytologically were all male. If these had been allowed to develop, the sex ratio might have been almost 1:1.

Table 2. Life history of the Béga Rapids colony, Beffa form of *Simulium damnosum* s.l.

Generation	Total adults (male:female)	No. of mated females	No. fed (refed)	Egg batches Fertile/Total	Egg batches reared	Fertile eggs in reared batches (No. hatched)
♀ parent collected as pupa	1	1	1	1/1	1	Not counted
F <sub>1</sub>	157(1.2:1)	15	10	2/2	2	230(?) 50(?)
F <sub>2</sub>	136(1.7:1)	24	18(1)	2/3	2	92(19)* 100(14)*
F <sub>3</sub>	6(1:1)	—	—	—	—	—

\* Most eggs produced larvae but failed to hatch.

collected as a pupa on March 19, 1982. One hundred and fifty-seven adults (85 males, 69 females and 3 escapes of unknown sex) were reared from the eggs laid by this female. Although 15 of the F<sub>1</sub> females mated and 10 took blood, only two survived to oviposit. They produced 230 and 50 fertile F<sub>2</sub> eggs, respectively. Both batches were reared and 136 adults were obtained including 50 females. Twenty-four F<sub>2</sub> females mated and 18 took blood but only three survived long enough to lay eggs. Two egg batches were fertile with 92 and 100 fertile eggs, respectively. All fertile eggs appeared to develop normally but only 33 hatched and only six adults were reared of which two escaped. The colony was lost when one of two reared males drowned after emergence and the other did not mate.

**MATING.** The males showed aggressive mating behavior while most females were refractory as reported by Simmons and Edman (1982) and Simmons et al. (1983). The mating sequence was also the same as that described by Simmons and Edman (1982) and similar observations were made of males frequently attempting to mate with each other. Mating activity was variable but often increased in the latter part of the day. Best results were normally obtained with 2-day old males not previously

exposed to other flies. The use of young females improved their chance of survival to blood feed and oviposit. Active males sometimes mated with two or more females in succession and some females mated a number of times. Consequently, the total number of mated females could not be ascertained simply by counting the number of apparently successful copulations. However, it was often possible to differentiate between females by means of size, abdominal extension etc. which facilitated the separation of inseminated flies.

**BLOOD-FEEDING.** Techniques using small cages or cloth sleeves for feeding many flies together on man or animals did not work with the Beffa form. The single tube method, although laborious and time consuming was much more successful (although Kaboua colony females were still difficult to feed). Five females were refed after oviposition (Tables 1 and 2) and one of these laid a second (infertile) egg batch. Feeding rates tended to be highest in the morning.

## DISCUSSION

Two populations of the Beffa form of *S. damnosum* s.l. were colonized on a small scale for the

Table 3. Fate of Kaboua colony, Beffa form of *S. damnosum* s.l., egg batches.

Generation	Egg batches	No. of eggs Fertile/Total (% fertile)	Fate of eggs
Parental ♀	a	426/447(95)	Reared (198 adults)
F <sub>1</sub>	a	Infertile (0)	No development
	b	211/244 (86)	Reared (35 adults)
	c	385/398(97)	Hatched but larvae died
F <sub>2</sub>	a	250/275(91)	Reared (22 adults)
	b	8/N.C.	Larvae died
F <sub>3</sub>	a	N.C.	Reared (9 adults)
F <sub>4</sub>	a	369/419(88)	Reared (43 adults)
F <sub>5</sub>	a	12/N.C.	12 eggs developed but none hatched.
	b	40/N.C.	Only 10 hatched and larvae died.
	c,d,e,f	Infertile (0)	No development.

N.C. = not counted.

first time in Africa. The high mating rate but reluctance to blood-feed shown by Beffa females from both the Mono and Okpara rivers agree with the finding of Simmons et al. (1983) and K. R. Simmons (personal communication) who worked on material from the same Mono River site. Nevertheless Simmons and Edman (1982) obtained high feeding and low mating rates with a Beffa colony from Senchi Rapids in the River Volta.

In spite of poor rearing results with Kaboua material and poor survival of Bégba Rapids adult flies, both colonies were maintained for several generations despite extreme inbreeding and no consistent shift in the sex ratio (Simmons and Edman 1982) was apparent. Nevertheless poor larval and adult survival, low egg fertility rates and low hatch rates, all occurred at some stage. Although these defects could have had many causes and often occur in first generation rearings, taken collectively they are quite suggestive of the detrimental effects of inbreeding.

Production of infertile egg batches by females thought to have mated, may have been partly due to the males mating more than once with the same female. At that time we were determining the number of inseminated females by the number of copulations observed. In addition, it is not known whether the males can inseminate more than one female in quick succession. Simmons and Edman (1982) observed that males mating twice in succession remained in copula for up to 20 min the second time.

The maintenance of the Kaboua colony for five generations in spite of the paucity of adults, proves that adult handling techniques are now adequate. Although multigenerational rearing on a very small scale has proved to be possible, large scale rearings are required to avoid inbreeding and to produce sufficient numbers for experimental purposes.

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