

*Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae)  
development: Rate, variation and the implications  
for forensic entomology

Jeffrey D. WELLS and Hiromu KURAHASHI\*

National Institute of Health, Department of Medical Entomology,  
Shinjuku-ku, Tokyo 162, Japan

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**Abstract:** The development of the Oriental latrine fly, *Chrysomya megacephala* (Fabricius), was examined at 27°C in order to resolve discrepancies in previous reports. Various developmental events were completed by all larvae by the following ages: egg hatch, 18 hr; first molt, 30 hr; second molt, 72 hr; pupariation, 144 hr; adult emergence, 234 hr. Differing descriptions of *C. megacephala* development appear to reflect variation in the length of the postfeeding period, during which time larvae may be particularly sensitive to environmental conditions. By sampling entire age cohorts, we were able to construct confidence bands about growth curves based on body length and dry weight. This allows the first measure of precision for estimates of larval age based on laboratory growth data.

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INTRODUCTION

The Oriental latrine fly, *Chrysomya megacephala* (Fabricius), has expanded its range tremendously in the last two decades. Formerly Australasian and Pacific in distribution, it is now widespread in Africa (Kurahashi, 1978; Prins, 1979; Braack, 1992; McGarry *et al.*, 1992) and the Americas (Baumgartner and Greenberg, 1984; Wells, 1991; Baumgartner, 1993).

*C. megacephala* is among the most pestiferous filth flies known, and is likely to transmit enteric pathogens and parasites under unsanitary conditions (Wells, 1991). This species also has a notable attraction to

fish, which it will infest (Esser, 1991; Olsen *et al.*, 1992).

Within developed countries, the greatest applied importance of *C. megacephala* may be during legal proceedings. Calliphorid larvae are useful in investigations of untimely death, since knowledge of their development rate may be used to determine the postmortem interval (Greenberg, 1991). *C. megacephala* commonly serves such a purpose in Hawaii (Goff and Odom, 1987), and because both the numbers of this species and the use of insects for forensic purposes are on the increase (Catts and Goff, 1992), the basic biology of this fly will assume even greater practical importance.

There have been conflicting descriptions of the development rate of *C. megacephala* as a function of temperature. Several authors

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\* Wells, J. D., 倉橋 弘: 国立予防衛生研究所昆  
虫医科学部 (〒162 東京都新宿区戸山 1-23-1)

gave incomplete descriptions of their rearing or sampling methods (Bohart and Gressitt, 1951; Khole, 1979; Subramanian and Mohan, 1980), making comparisons between results somewhat difficult. They suggest, however, an oviposition to pupariation period of 5-6 days at 25-29°C. In a more exactly described study, Goodbrod and Goff (1990) reported an oviposition to pupariation period of 5.5 to 7 days (depending on larval density), at 23.5°C. In another exactly described experiment, however, Nishida (1984) observed oviposition to pupariation periods of 12 days at 24°C, and 11 days at 30°C. This study was an attempt to resolve this apparent discrepancy concerning the development of a medically and forensically important blow fly.

#### MATERIALS AND METHODS

*Larval growth.* Our colony of *C. megacephala* was descended from a female collected in Bangalore, India, in 1993, and had been maintained in the laboratory for six generations at the start of this study. Eggs considered to be the same age were obtained during a 2 hr period at 27°C. For each rearing container (see below) we selected what appeared to be a batch of eggs from a single female based on the number of eggs (est. 150-300) and their parallel arrangement. Each container was held under light conditions of 16L:8D at 27±0.5°C, a temperature within the range of those used by previous investigators (Bohart and Gressitt, 1951; Khole, 1979; Subramanian and

Table 1 Proportion *C. megacephala* developmental stages according to each sample age at 27°C.

Age (hr)	Egg	Larval instars			Puparium	Adult	n
		First	Second	Third			
12	1.0						—
18		1.0					139
24		1.0					137
30			1.0				73
36			1.0				35
42			1.0				78
48			0.92	0.08			258
60			0.31	0.69			59
72				1.0			206
84				1.0			65
96				1.0			144
108				1.0			155
120				0.90	0.10		62
144					1.0		89
198*					1.0		
210					0.41	0.59	
222					0.15	0.85	
234						1.0	166**
Total							1,666

Ages 12 through 144 hr represent containers in which all individuals were removed at the time of sampling. Ages 198 through 234 hr represent two containers from which adults were removed when first seen. \* Earlier observations of jars prepared for adult emergence not shown. \*\* Total for the two jars used for adult emergence.

Mohan, 1980, Nishida, 1984; Goodbrod and Goff, 1990). During sampling all larvae from a container were removed and killed in boiling 70% ethanol. The sample ages were selected in advance (Table 1), and containers were prepared in an order determined by lottery. Younger larvae were more intensively sampled (Table 1) in order to more precisely determine the age at molting. Containers for samples up to age 36 hr consisted of a 250 ml plastic cup with a perforated lid and 40 g pork liver. Older larvae were reared in a 1,500 ml glass jar, lined with a plastic bag filled with 20 g sawdust and 100 g pork liver, and sealed with filter paper. Instar (indicated by the number of spiracular slits (Smith, 1986)), wet body length and dry weight (Shimadzu Chemical Balance, Kyoto) were recorded for each larva. Larvae were dried for 72 hr at 60°C if the samples contained third instars (Table 1), or for 48 hr if they did not. The youngest larvae were too small to be weighed as individuals on a balance that read to 0.1 mg, and mean weights were calculated from groups of the following sizes for each age: 18 hr,  $n=45$ ; 24 hr,  $n=45$ ; 30 hr,  $n=35$ ; 36 hr,  $n=10$ ; 42 hr,  $n=10$ . Because these numbers did not divide evenly into the total samples for each age (Table 1), a few larvae were measured for body length but not weight.

**Adult emergence.** Two 1,500 ml jars were prepared in the above manner, but were filled to the top with sawdust. Holes were cut in the center of the filter paper so that newly emerged adults could escape into a plastic container. Adult flies were removed and sexed every 12 hr.

## RESULTS

A total of 16 rearing containers and 1,666 individuals were sampled. Table 1 displays the occurrence of each development stage according to sample age. The unhatched eggs observed at 12 hr were not counted, but were estimated to number at least 150. Samples at ages 18 and 30 hr included a small number ( $\leq 3\%$  of total sample) of

eggs that were unembryonated, and presumed to be non-viable. These individuals were not included in Table 1.

Hatching and the molt from first to second instar were highly synchronized events, since samples taken every 6 hr did not detect populations of mixed stages. In contrast, variation was detected in the timing of subsequent molts, pupariation and emergence. The maximum possible range in age for these events may be estimated from the time separating the oldest sample with 100% of a particular instar from the youngest sample with 100% of the next instar. According to this definition, the following ranges are possible: second to third larval instar, 30 hr; third instar to puparium, 36 hr; puparium to adult, 36 hr. Caution should be taken when comparing these figures since the frequency of observation was not consistent for all ages.

### Larval growth

Figures 1 and 2 show the change in *C. megacephala* body length and weight as a function of age. The s-shaped curve is typical for the larval development of calliphorids, and reflects the fact that the third instars grow rapidly at first, and then cease to feed (Greenberg, 1991).

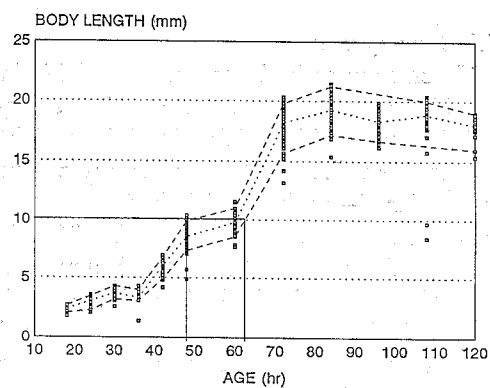


Fig. 1 Body length of *C. megacephala* larvae reared at 27°C.

The dotted line connects the means, and the dashed lines enclose 95% of the observations. The solid lines illustrate the technique for calculating age based on body length (see DISCUSSION).

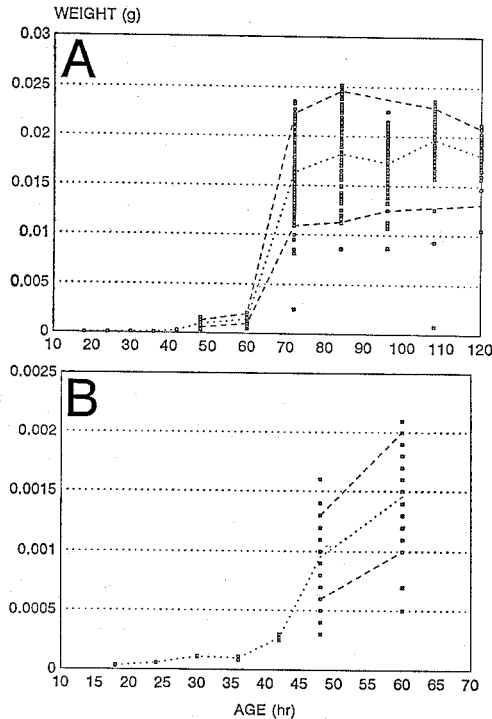


Fig. 2 Dry weight of *C. megacephala* larvae reared at 27°C.

The dotted line connects the means and the dashed lines enclose 95% of the observations (not possible for younger samples, see text). A: entire curve. B: earlier section of curve expanded for greater resolution.

#### Adult emergence

No emergence had occurred as of 198 hr, but adults were observed in both jars at age 210, 222 and 234 hr (Table 1). Males slightly preceded females. Mean sample ages were: jar 1—males 217.5 hr ( $n=16$ ), females 220.4 ( $n=22$ ); jar 2—males 214.8 hr ( $n=62$ ), females 217.2 ( $n=62$ ). Large numbers of puparia in both jars, *i.e.*, roughly one half the number of adults produced, failed to complete development. These were not included in Table 1.

#### DISCUSSION

Our results resemble those of most authors (Bohart and Gressitt, 1951; Khole, 1979; Subramanian and Mohan, 1980; Goodbrod

and Goff, 1990), and are rather different from those of Nishida (1984). In particular, the differences occurred in the postfeeding period, *i.e.*, the *C. megacephala* observed by Nishida pupariated about 1 week after reaching maximum size at 24 and 30°C, compared to at most 2.5 days reported here. There is little reason to suspect geographic variation as the cause of such a difference. Although only Nishida's colony originated from near Okinawa, there is general agreement between studies of populations from Hawaii (Goodbrod and Goff, 1990), Guam (Bohart and Gressitt, 1951), two sites in India (Subramanian and Mohan, 1980; our results), and a population whose origin was not given (Khole, 1979).

In our experience blow fly larvae can delay pupariation if conditions are (we presume) sub-optimal, *e.g.*, larvae have no shelter or are very wet (see also Ohtaki, 1966), and this flexibility might explain the discrepancy between studies of *C. megacephala* development. The potential variation in the duration of the postfeeding stage indicates that special attention must be given to environmental conditions when estimating the age of postfeeding larvae.

Several researchers have investigated the influence of environmental conditions on carrion fly growth (Catts and Goff, 1992). Few authors, however, provide any indication of the full variation in size among fly larvae of the same age. Reiter (1984) sampled entire age cohorts of *Calliphora vicina* Robineau-Desvoidy, but did not specify the effect of variation on estimates of maggot age. We believe that this is the first study to describe the variation in entire laboratory age cohorts rather than that of sub-samples, and without such data it is difficult to establish the precision of larval-age estimates.

Statistical methods for estimating larval age from such curves are in preparation (Wells and LaMotte, unpublished), but a less sophisticated technique works nearly as well. The dashed lines in Figs. 1 and 2 contain 95% of the observations, and may serve as crude 95% confidence bands for larval growth. If these curves were con-

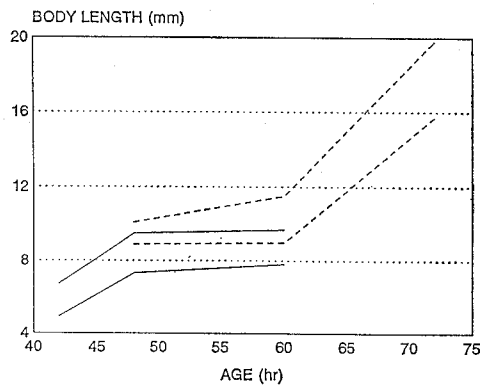


Fig. 3 Separate 95% confidence bands for second (solid lines) and third (dashed lines) instar *C. megacephala* from Fig. 1.

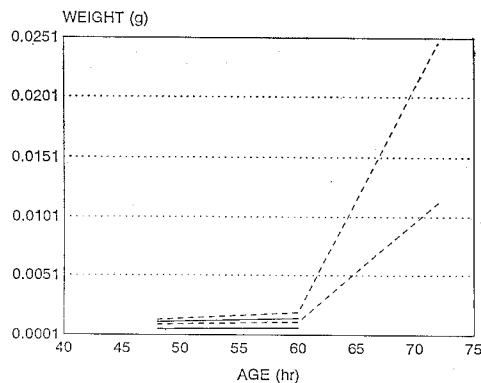


Fig. 4 Separate 95% confidence bands for second (solid lines) and third (dashed lines) instar *C. megacephala* from Fig. 2.

sidered an appropriate model of growth for a larva of unknown age, a horizontal line from a body length or weight value would intersect the dashed lines at two points defining a 95% confidence interval about that larva's age (see solid line in Fig. 1). Using this approach, it is clear that estimates of age based on either measurement variable are most precise where the curve is most steep. The value of the weight data was compromised by the fact that the smallest larvae could not be individually measured. Because the full range of weights could not be shown, a confidence band was not drawn for this portion of the curve. This difficulty could be surmounted in future studies by the

use of a more sensitive balance.

Confidence bands were constructed separately for second and third instars in the regions of Figs. 1 and 2 where they were mixed (Figs. 3 and 4). Third instars were clearly larger than seconds, and some greater precision in estimating age from length or weight is possible when instar is also considered.

Forensic entomologists often rely on specimens collected and preserved using a variety of techniques by police or medical examiners. It has been shown that the kind of preservative fluid may influence the body length of a preserved larva (Tantawi and Greenberg, 1993), and it seems likely that freezing could have a similar effect. Dry weight, therefore, may be less affected than body length by preservative fluids, and therefore more useful in situations where larvae have not been prepared in the same manner as in laboratory studies.

Instar data were more effective than size for estimating the age of young larvae. Most larvae age 72–120 hr could not be distinguished on the basis of either size or instar. Greenberg (1991) described the decrease in crop size that occurs during the postfeeding larval period, and how this is of some use in aging full-size larvae.

One striking result was the presence of individuals that were much smaller than the mean (Figs. 1 and 2). Such stunted individuals have not been reported before, perhaps because few investigators sample entire cohorts of larvae. A similar skewness to the left (Sokal and Rohlf, 1981) in the distribution of weights has been observed in other species (unpublished), and this may be a general characteristic of larvae reared in the laboratory. In this study, extremely small larvae were so rare that they had little effect on the width of the confidence bands. For that reason, they created no problem for estimating the age of a larva according to the level of probability commonly used in science, *e.g.*, one may be 95% sure that a 10 mm larva reared under these conditions is 49–63 hr old (Fig. 1) even though a few of this size were much older. More strict

standards, however, may be applied in court.

We suggest that studies of the influence of environmental conditions, such as temperature (Nishida, 1984) or drugs (Goff *et al.*, 1993), on larval growth include explicit consideration of changes in the variance as well as the mean. It is reasonable to believe that the variation in larvae of the same age under these controlled conditions represents a minimum of what might be found in the field. It is also possible that some species of carrion larvae may yield a more precise estimate of the postmortem interval than other species, and so would be more valuable in forensic investigations.

Our sample sizes and direct observation of dead eggs and puparia indicate that the *C. megacephala* in some jars experienced high mortality. We do not know if this was an artifact of our methods. This does, however, identify a potential problem for the forensic entomologist. Dead larvae can be recognized in the field, and decay relatively rapidly. Dead eggs or puparia could easily go unrecognized if they were preserved in fluid or frozen by investigators. Obviously the age of such individuals could not be estimated in the manner used for live eggs or puparia. Such evidence would be particularly misleading in situation where a very limited sample was available. The senior author was consulted for a murder trial in which the entomological evidence consisted of three calliphorid eggs that were preserved during an autopsy that was performed years before (State of Florida *vs.* Sean Patrick Esty). In that instance the eggs were fully embryonated, and so apparently had been viable at the time they were preserved. Investigation of the incidence of egg and puparium mortality in wild populations of carrion flies is warranted.

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#### 摘 要

オビキンバエ (双翅目: クロバエ科) の  
法医学昆虫学への利用:

幼虫の発育速度と体長および体重の変異

ニューギニア原産のオビキンバエ *Chrysomya megacephala* (Fabricius) は、東洋区に広く分布している衛生上重要な汚物バエである。近年、その分布はアフリカ熱帯区、新熱帯区、新、旧北区などに広がりがつある。不衛生な地域では衛生上要注意の種類である。いっぽう、死体にも発生する本種は都市などでは犯罪に関わる法医学上の重要性も増してきた。死亡時間を推定するうえで正確な発育速度を知る必要性が高まってきた。本研究はインドのバンガロールで採集された1雌由来の飼育コロニーを用いて27°Cにおける幼虫の発育速度と体長および体重の変異を調べたものである。囲蛹化完了までに144時間、成虫羽化完了には234時間要した。従来の方法と異なり、全バッチ個体群をサンプルすることにより体長と乾燥重量について95%信頼限界を示す成長曲線を得た。