J Forensic Sci, Nov. 2002, Vol. 47, No. 6
Paper ID JFS2002119_476
Available online at: www.astm.org

Martin Grassberger,[1,2] *M.D., M.Sc. and Christian Reiter*,[1] *M.D.*

Effect of Temperature on Development of *Liopygia* (= *Sarcophaga*) *argyrostoma* (Robineau-Desvoidy) (Diptera: Sarcophagidae) and Its Forensic Implications

ABSTRACT: The temperature-dependent development of the forensically important flesh fly *Liopygia* (*Thomsonea*) *argyrostoma* (Robineau-Desvoidy) (=*Sarcophaga argyrostoma*) was studied at six constant temperature regimes in the laboratory. Total developmental time of *L. argyrostoma* from larviposition to adult emergence was 54.9 ± 1.45, 31.3 ± 1.1, 22.2 ± 0.67, 16.3 ± 0.54, and 14.9 ± 0.4 days (±S.D.) when reared at 15, 20, 25, 30, and 35°C, respectively. At 8°C, larval development was not completed. From linear regression of development rates of five of the six studied constant temperature regimes, it was determined that the minimum development threshold (t_L) for total immature development is 7.4°C, and the overall thermal constant (K) for *L. argyrostoma* is 396.4 ± 19.18 (mean ± S.D.) day-degrees (DD) above the threshold.

KEYWORDS: forensic science, *Liopygia argyrostoma*, *Sarcophaga argyrostoma*, Sarcophagidae, forensic entomology, postmortem interval, myiasis, development time, thermal constant

The larvae of sarcophagid flies (commonly called "flesh flies") potentially are among the most useful insects for investigation of suspicious human death (1). *Liopygia argyrostoma* (Robineau-Desvoidy) (=*Sarcophaga argyrostoma*) was originally described in 1830 and occurs in all zoogeographical regions (except Australia and New Zealand) due to its culturophily and synanthropy (2). In northerly latitudes, the species occurs in association with human activity. Povolny and Verves, in their comprehensive work on "The Flesh-Flies of Central Europe" (2), placed *L. argyrostoma* in the separate genus *Liopygia* Enderlein, 1928, and in the subgenus *Thomsonea* Rohdendorf, 1937. However, Pape (3) has restored the older concept of *Sarcophaga*, placing all these divisions as subgenera, preferring broader genera and recognizing that half of the divisions of *Sarcophaga* worldwide are monospecific, which is strongly indicative of the need for a more critical appraisal. According to Pape (3), flies of this species belong to the genus *Sarcophaga* Meigen and to the subgenus *Liopygia* Enderlein. The nomenclature used in the present study follows the classification of Povolny and Verves (2), because we used their keys for identification of the adult flies, but it should be noted that the developmental data reported also apply to *Sarcophaga argyrostoma*. For a detailed discussion on a DNA-based identification and molecular systematics of forensically important Sarcophagidae see Ref 1.

The females of *L. argyrostoma* (like almost all *Sarcophagidae*) usually deposit 1st instar larvae instead of eggs (i.e., they are larviparous = viviparous). However, during the work described here, we observed some cases of oviposition in laboratory-reared fly colonies. The adult flies visit decaying substrates, faeces, and carcasses and also feed at flowers (2). Larvae normally develop in decaying meat but are also known as predators and parasitoids of various animals (2,4,5).

Flies belonging to the family *Sarcophagidae* have received much attention due to their myiasis potential and vector for pathogens (6). *L. argyrostoma* has been reported several times in recent years as an agent of human cutaneous (wound) and genitourinal (vaginal) myiasis (7–12). Recently, attention has been focused on the *Sarcophagidae* because of their use in medicocriminal entomology (1,2,5,13–18). Benecke (18) found *L. argyrostoma* (*Parasarcophaga argyrostoma*) larvae on a corpse lying on the balcony of a flat in the eighth floor in Cologne, Germany, for 25 days. In Belgium, Leclercq (5) reported this species from a cadaver in an advanced state of putrefication. Zohdy and Morsy (11) studied larval and pupal development of *L. argyrostoma* (*Parasarcophaga argyrostoma*) at 20, 25, and 30°C in Cairo, Egypt. However, no detailed developmental data are available for *L. argyrostoma* from temperate regions describing development under a broader temperature range.

Due to its high abundance in Austria and other parts of the world and the frequency with which *L. argyrostoma* appears in cases of human myiasis and death, detailed developmental data are essential to estimate time since infestation or death. Our study will extend the use of *L. argyrostoma* in legal investigations and allow a more precise estimate of the postmortem interval to be made when this species is recovered from the death scene. Additionally, in cases of infested bedsores or similar conditions, the time of personal or medical negligence can be estimated.

Material and Methods

Larvae of *L. argyrostoma* were collected from human cadavers in the morgue of the Institute of Forensic Medicine, University of

[1] Institute of Forensic Medicine, University of Vienna, Sensengasse 2, A-1090 Vienna, Austria.
[2] Institute of Anthropology, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria.

Received 6 April 2002; and in revised form 29 May 2002; accepted 29 May 2002; published 2 Oct. 2002.

Vienna, from June to August 2001 and reared to adult stage on beef liver. Adults were examined under the binocular microscope and identified using the morphological characters given by Povolny and Verves (2), including preparation of the male genitalia. The identification was confirmed by Dr. Richard Zehner (Institute of Legal Medicine, Frankfurt/Germany) using molecular markers of the COI and COII regions derived from original specimen kindly provided by Dalibor Povolny (Institute of Zoology, G. Mendel University Brno, Czech Republic).

Flies were held in an insectary at 22 to 25°C with approximately 60% RH and a photoperiod of 14:10 (L:D) hours. About 300 adult flies were kept in screen cages (40 by 30 by 30 cm) and fed a mixture of dry granular sugar, powdered milk, and brewer's yeast. Water was supplied by inversion of a beaker on a Petri dish covered with a filter paper.

Growth under Different Constant Temperature Regimes

Larvae were collected within 30 min of larviposition, using black 35-mm filmcups baited with decaying beef liver. We found this a satisfying procedure for collection of larvae, since the female adults seemed to be attracted by the dark and moist environment thus created. About 100 larvae were transferred to 250 g raw beef liver, cut in approximately 1-cm-thick slices, and subsequently transferred into plastic jars (25 by 25 by 7 cm) covered with a gauze net. Using this procedure, we achieved a more two- dimensional and disseminated feeding behavior, which is essential to prevent maggot mass formation. The bottom of the jars was covered with sawdust to provide a dry place for pupation. This is important because it is considered that larvae could delay pupation under suboptimal conditions (19). The jars were then placed into an environmental chamber (KBK/LS 4330, Ehret, Germany) at one of the six desired temperature regimes (8, 15, 20, 25, 30, and 35° C, respectively), with a relative humidity of 60 ± 5% and constant darkness. This procedure was repeated ten times for each temperature regime.

Three of the largest maggots were removed from the plastic jars every 12 h. When the first maggots stopped feeding, those in the migratory phase were removed for measurement purposes until 10% of the maggots underwent pupation. Measuring the oldest individuals (i.e., the largest prior to peak feeding) is regarded as common practice in forensic entomology. After peak feeding, as the maggots prepare for pupation and decrease in total body length, age can be based on relative length of total body compared to visible crop length. Twice a day we recorded the mean temperature within the center of actively feeding maggots using a digital thermometer (MD 3150 Beckmann + Egele, Germany).

Specimens were killed in boiling water to prevent shrinkage, as might be the case with other killing and preservative solutions (20). Measurement was followed immediately under a binocular microscope in 0.1-mm units using a vernier caliper.

Developmental Threshold and Thermal Constant

Lower threshold (t_L) for development was estimated from the linear regression (Microcal Origin® 6.0) of the developmental rates (y = 1/developmental time) on constant temperature (x) (21,22). The thermal constant K was calculated from the equation $K = y(t - t_L)$, where y is the developmental time (days), t is the rearing temperature (°C), and t_L is the theoretical lower developmental threshold temperature (°C). The thermal constant was calculated for temperatures 15, 20, 25, 30, and 35°C (for larval development and total immature development) to obtain the overall K (mean ± SD) required for stage completion. Values of K represent the number of degree days (DD) above the threshold (t_L) needed for larval development and total immature development.

Results

Growth Curves from Constant Temperature Regimes

Means of the maximum measured lengths of all rearings were plotted against time for each of the constant temperature regimes (Fig. 1). The mean minimum duration of development (±S.D.)

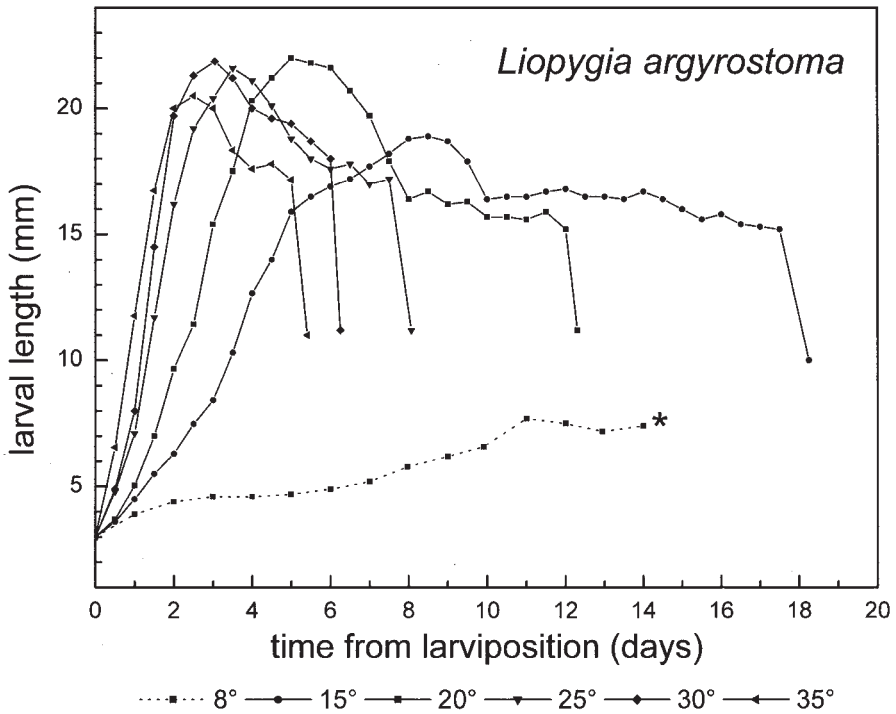

FIG. 1—*Development of L. argyrostoma from larviposition to pupariation at six different constant temperature regimes (means of the maximal measured lengths).*

TABLE 1—*Minimal duration and calculated degree-days of larval and total immature development.*

Temperature (°C)	Larviposition to Pupariation		Larviposition to Adult Emergence	
	Days (mean ± S.D.)	Degree-days† (DD)	Days (mean ± S.D.)	Degree-days‡ (DD)
8	…*	…	…*	…
15	18.3 ± 0.72	142.7	54.9 ± 1.45	417.2
20	12.3 ± 0.36	157.4	31.3 ± 1.1	394.4
25	8.1 ± 0.31	144.2	22.2 ± 0.67	390.7
30	6.3 ± 0.19	143.6	16.3 ± 0.54	368.4
35	5.4 ± 0.22	150.1	14.9 ± 0.4	411.2
Overall *K* (mean ± S.D.)		147.6 ± 6.2		396.4 ± 19.18

* No data available for calculation of thermal constant (*K*).
† Developmental minimum used for DD calculation: 7.2°C.
‡ Developmental minimum used for DD calculation: 7.4°C.

TABLE 2—*Average stage duration of L. argyrostoma based on first 10% of population to enter and complete the respective life stage under six temperature regimes.*

Temp. (°C)	Stage Duration (h)			
	1st Instar	2nd Instar	3rd Instar	Pupa
8	102	215	…*	…*
15	41	43	355	879
20	24	26	245	456
25	14	16	164	339
30	12	14	125	240
35	12	12	106	228

* Larvae did not complete development at 8°C.

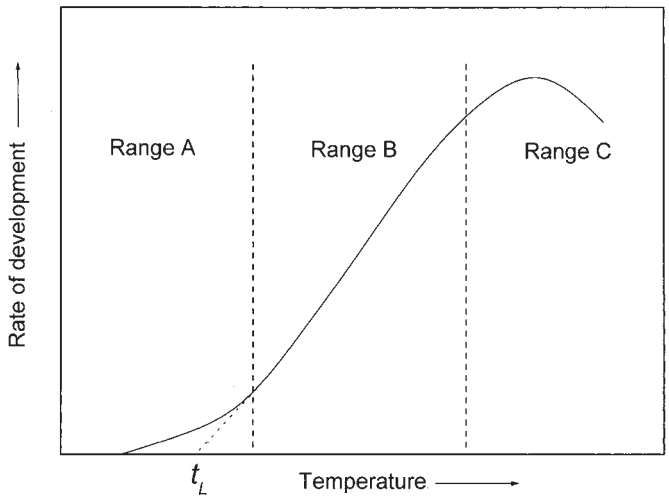

FIG. 2—*Generalized relationship between the rate of development and temperature, showing the non-linear portions A and C and the linear portion B used to estimate the minimum development threshold (t_L) by extrapolation (after Ref 21).*

from larviposition to pupariation and from larviposition to adult emergence (total immature development) at each of the six studied temperature regimes is given in Table 1. Development time from oviposition to adult emergence was shortest (mean 14.9 ± 0.4 days) at 35°C and longest (mean 54.9 ± 1.45 days) at 15°C. At 8°C the larvae did not complete development and frequently died between the 13th and 19th day. In the center of actively feeding 2nd and 3rd instars of the temperature regimes 15, 20, and 25°C, the recorded temperature was sporadically 0.5 to 1.8°C above the desired temperature. Stage duration time was recorded after 10% of the sample population completed development from the previous stage (Table 2).

Developmental Threshold and Thermal Constant

The development of poikilotherms such as *L. argyrostoma* is widely described using the temperature summation model, which is valid for the linear proportion (Range B) of the sigmoidal development curve (Fig. 2) (21). Between 15 and 35°C, development of *L. argyrostoma* was linearly related to temperature ($r = 0.99$, $P < 0.001$).

The degree-days at 15 to 35°C were essentially the same, indicating no developmental delays because of stress in this range. However, at 8°C larvae developed considerably slower than at 15°C and did not complete development, indicating stress. Therefore, the rate of development at 8°C was not included in linear regression analysis.

The rate of total immature development (1/days) increased with temperature, with development rates of 0.01821, 0.032, 0.045, 0.06135, and 0.06711 at 15, 20, 25, 30, and 35°C, respectively, and $r = 0.99$, $P < 0.001$; $y = 0.00254x - 0.01884$. From the regression line plotted in Fig. 3, it was determined that the minimum development threshold (t_L) for total immature development was 7.4°C, and the calculated overall thermal constant (K) for *L. argyrostoma* was 396.4 ± 19.18 (mean ± S.D.) day-degrees above the threshold.

Linear regression of developmental rates from larviposition to pupariation resulted in a minimum development threshold of 7.2°C and a calculated thermal constant for larval development of 147.6 ± 6.2 (mean ± S.D.) day-degrees above the threshold.

The larval and pupal periods were related inversely to temperature and were about 35 and 65% of the total developmental period between 15 to 35°C.

Discussion

This study is in agreement with the results reported by Zohdy and Morsy (11) only at a temperature of 30°C. Zohdy and Morsy reported a substantially longer developmental duration for larval and pupal stages at 20°C than what was produced with our laboratory rearings. At a constant temperature of 20°C, *L. argyrostoma*

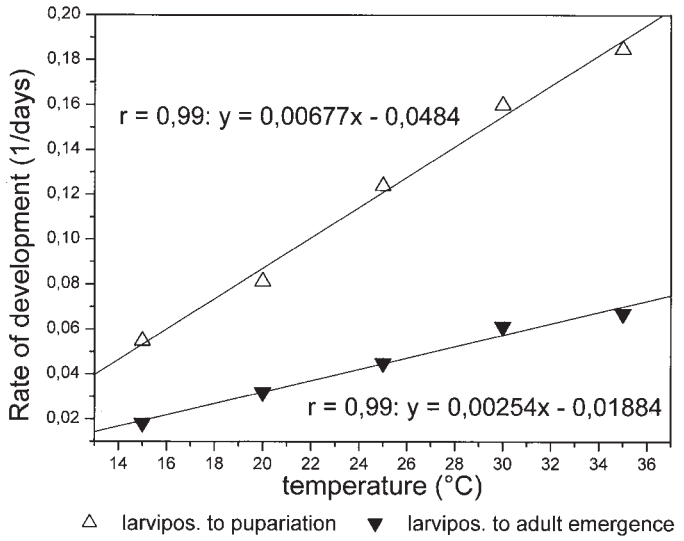

FIG. 3—*Linear regression of rearing temperature and rate of development (from larviposition to pupariation and from larviposition to adult eclosion) of L. argyrostoma.*

from Egypt required 41.4 days for total immature development (compared to 31.3 days in present study), indicating poor cold adaptation. Whether the observed differences have to be attributed to different experimental conditions (e.g., food source) remains unclear. Saunders (23), rearing *L. argyrostoma* (*S. argyrostoma*) under different light cycles, found that the length of larval development varies with the period of the environmental light cycle. The largest observed difference in mean developmental time between larvae from various photoperiods was about three days (20%). To our knowledge, no study has directly addressed the question of light cycle and its influence on larval development of forensically important fly larvae.

However, larger differences in development times do not necessarily have to be attributed to variation in experimental method (extrinsic factors). Geographic adaptation (intrinsic factors) could explain a difference in temperature-dependent development. The ideal experiment to clarify this issue would be rearings of specimens from several geographic regions under the same experimental conditions.

In addition, published standard requirements in experimental conditions regarding light cycle, temperature regime (constant versus cycling temperatures), food source, and the number of maggots per weight of rearing substrate would help to make data of various studies comparable.

Goff et al. (24) observed a more rapid development of *Parasarcophaga ruficornis* (Fabricius) larvae when reared on tissues from rabbits containing 3,4-methylenedioxymethamphetamine. Whether *L. argyrostoma* exhibits a similar acceleration in larval growth when feeding on decomposing tissues containing amphetamine derivates is unknown. However, until otherwise proven, a similar increase of larval development rate has to be anticipated when estimating the post-mortem interval in forensic entomological casework.

There is a continuing need to refine and improve developmental data of forensically important insect species. Precise values for developmental minima and degree-day estimates by stage are important areas of improvement (25). Additionally, as the present paper shows, studies characterizing variation in these parameters between geographically distinct populations of the same species would be of great value for future forensic entomological casework.

Several forensically important Calliphoridae require considerable less degree-days for total immature development compared to 396.4 ± 19.18 (as in Table 1) (mean ±S.D.) degree-days for *L. argyrostoma* (e.g., *Phaenicia sericata*: 197 ± 27, *Phormia regina*: 202 ± 33, *Chrysomya rufifacies*: 158 ± 18) (25), making it a good indicator for longer postmortem intervals when recovered from the scene.

In addition, our study demonstrated that *L. argyrostoma* could be reared successfully in the laboratory. A crucial detail, when specimens recovered from a corpse should be reared in the growth cabinet to pinpoint time since death.

Acknowledgments

This work was supported in part by a grant of the Mayor of the City of Vienna. We also wish to thank Dr. Richard Zehner (Institute of Legal Medicine, Frankfurt/Germany) for confirming our identification of *L. argyrostoma* using molecular markers and Dr. Jens Amendt for useful comments on sarcophagid nomenclature.

References

1. Wells JD, Pape T, Sperling FAH. DNA-based identification and molecular systematics of forensically important sarcophagidae (Diptera). J Forensic Sci 2001;46(5):1098–102.
2. Povolny D, Verves Y. The flesh-flies of Central Europe (Insecta, Diptera, Sarcophagidae). Spixiana 1997;Suppl 24:217–8.
3. Pape T. Catalogue of the Sarcophagidae of the world (Insecta: Diptera). Memoirs on Entomology, International 1996;8.
4. Aldrich JM. Sarcophaga and allies in North America. Thomas Say Foundation, La Fayette 1916;50(1):1–302.
5. Lecqlerq M. Entomologie et medicine légale: *Sarcophaga argyrostoma* Rob.-Desv. (Dipt., Sarcophagidae) et *Phenicia sericata* Meig. (Dipt., Calliphoridae). Bull Ann Soc Rent Belg 1976;112(4):119–26.
6. Greenberg B. Flies and disease. Vol. II: Biology and disease transmission. Princeton (NJ): Princeton University Press, 1971.
7. James MT. Flies that cause myiasis in man. U.S. Dept Agric Misc Publ 1947;631:1–175.
8. Burgess NR. A case of human myiasis in London. Trans R Soc Trop Med Hyg 1966;60:432–3.
9. Aspöck H, Leodolter I. Vaginale Myiasis durch *Sarcophaga argyrostoma*. Wien Klin Wochenschr 1970;82:518–20.
10. Wenger R, Hinaidy HK. Über Wundmyiasis des Menschen in Österreich. Wien Klin Wochenschr 1979;91:378–80.
11. Zohdy NZ, Morsy LE. On the biology of the grey flesh fly, *Parasarcophaga argyrostoma* (Robineau-Desvoidy). J Egypt Soc Parasitol 1982;12:85–95.
12. Burgess I, Spraggs PDR. Myiasis due to Parasarcophaga argyrostoma—first recorded case in Britain. Clin Exp Dermatol 1992;17:261–3.
13. Smith KGV. A manual of forensic entomology. British Museum (Natural History), London and Cornell University Press, Ithaca, NY, 1986.
14. Introna FJ, Suman TW, Smialek JE. Sarcosaprophagous fly activity in Maryland. J Forensic Sci 1991;36:238–43.
15. Byrd JH, Butler JF. The effects of temperature on *Sarcophaga haemorrhoidalis* (Diptera: Sarcophagidae) development. J Med Entomol 1998;35:694–8.
16. Introna F, Campobasso CP, Di-Fazio A. Three case studies in forensic entomology from southern Italy. J Forensic Sci 1998;43:210–4.
17. Byrd JH, Castner JL. Forensic entomology. The utility of arthropods in legal investigations. Boca Raton: CRC, 2001;287–302.
18. Benecke M. Six forensic entomology cases: description and commentary. J Forensic Sci 1998;43:797–805.

19. Wells JD, Kurahashi H. *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae) development: rate, variation and the implications for forensic entomology. Jpn J San Zool 1994;45(2):303–9.
20. Tantawi TI, Greenberg B. The effect of killing and preservative solutions on estimates of maggot age in forensic cases. J Forensic Sci 1993;38:702–7.
21. Campbell A, Frazer BD, Gilbert N, Gutierrez AP, Mackauer M. Temperature requirements of some aphids and their parasites. J Appl Ecol 1974;11:431–8.
22. Tun-Lin W, Burkot TR, Kay, BH. Effects of temperature and larval diet on development rates and survival of the dengue vector *Aedes aegypti* in north Queensland, Australia. Med Vet Entomol 2000;14:31–7.
23. Saunders DS. Circadian control of larval growth rate in *Sarcophaga argyrostoma*. Proc Nat Acad Sci USA 1972;69:2738–40.
24. Goff ML, Miller ML, Paulson JD, Lord WD, Richards E, Omori AI. Effects of 3,4-methylenedioxymethamphetamine in decomposing tissues on the development of *Parasarcophaga ruficornis* (Diptera: Sarcophagidae) and detection of the drug in postmortem blood, liver tissue, larvae and puparia. J Forensic Sci 1997;42(2):276–80.
25. Higley LG, Haskell NH. Insect development and forensic entomology. In: Byrd JH, Castner JL, editors. Forensic entomology. The utility of arthropods in legal investigations. Boca Raton: CRC, 2001;287–302.

Additional information and reprint requests:
Martin Grassberger, M.D., M.Sc.
Medical and Forensic Entomology
Institute of Forensic Medicine
University of Vienna
Sensengasse 2, A-1090 Vienna
Austria