An Artificial Diet for Larvae of *Calosoma sycophanta* (Coleoptera: Carabidae), a Gypsy Moth (Lepidoptera: Lymantriidae) Predator[1]

Ronald M. Weseloh

Department of Entomology, Connecticut Agricultural Experiment Station, New Haven, CT 06511 USA

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Abstract A newly-developed larval diet for *Calosoma sycophanta* L. consists of beef liver and chicken meat. Larvae reared individually on this diet develop at about the same rate and have the same survival as those fed gypsy moth pupae, *Lymantria dispar* L., the usual prey of the beetle. However, adults of diet-reared larvae were significantly smaller than adults of larvae reared on gypsy moth pupae. When reared in groups on the diet, beetles were significantly smaller and did not survive as well as those fed gypsy moth pupae. This diet, or a variation thereof, may be useful as part of a program to rear the beetle inexpensively.

Key Words Coleoptera, Carabidae, *Calosoma sycophanta,* Lepidoptera, Lymantriidae, *Lymantria dispar,* gypsy moth, artificial diet, predator

Calosoma sycophanta L. is an important predator of outbreak populations of the gypsy moth, *Lymantria dispar* L., in North America (Bess 1961, Campbell 1975, Weseloh 1985a, b, 1990). In the last several years, there has been interest in releasing this predator in areas of North America where the gypsy moth has recently invaded (Weseloh et al. 1995). As part of an effort to increase the availability of this insect for such releases, a procedure was developed to rear the cannibalistic larvae of *C. sycophanta* in groups (Weseloh 1996). However, this method required that the larvae be fed gypsy moth pupae which are expensive to rear. An artificial diet that would make it unnecessary to rear prey could greatly increase the efficiency of rearing *C. sycophanta.* This manuscript describes a study in which such a diet for larval beetles was developed.

Materials and Methods

Calosoma sycophanta beetles were originally collected from various forests in Connecticut in 1982-4, and their progeny, used in this study, have been reared in the laboratory since by methods described in Weseloh (1996). Adult beetles are long-lived and hibernate in soil during the winter. Larval beetles were obtained by removing adults from storage in moist peat moss at 5°C, and placing male-female pairs in plastic Petri dishes (2.5 × 14 cm) containing a 12.5-cm diam circle of filter paper in the bottom and a 3.5 by 1-cm diam glass vial with moist, rolled filter paper. The adults were fed gypsy moth larvae until mating was observed or the female began to ovi-

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posit. The pairs were then placed in glass jars (9.5 by 9 cm) that were about 1/4 full of moist peat moss and fed gypsy moth larvae. The females oviposited in the peat moss, and each day the eggs were removed and placed in plastic Petri dishes (1.5 by 9 cm) with a circle of 9-cm diam filter paper in the bottom that was wet almost to saturation. Eggs were held under these conditions until larvae eclosed. Except as noted, larvae were reared individually by placing each in a separate 1.5 by 9 cm diam plastic Petri dish containing a circle of 9-cm diam filter paper in the bottom. The filter paper was slightly moistened when a larva was placed in the dish, but not thereafter. Larvae were reared either by providing them daily with several gypsy moth pupae or one of the experimental diets. Filter paper and diet were changed daily. Larval molting times and mortality were recorded. *Calosoma sycophanta* larvae have three instars. When feeding was completed in the third stadium, each larva was placed in a 30-ml plastic creamer cup, each about 1/2 full of moist peat moss. Times of molting to the pupal and adult stages, as well as any additional mortality, were recorded. The maximum length of the elytra and maximum width of the pronotum of all adult beetles also were measured.

Preliminary tests showed that raw beef liver may be an adequate diet for larvae. Thus, the candidate artificial diets used were: raw beef liver, raw calf liver, raw ground chicken meat (chicken burger), raw beef hamburger, raw pork meat, canned tunafish, and canned catfood (9-Lives Salmon Supreme®, Heinz® Pet Products, Newport, KY). These were all purchased at a local grocery store. Unless otherwise noted, the candidate diets were cut into approximately 1-cm cubes, wrapped individually in 5-cm squares of plastic wrap, and frozen until needed. A piece of one of these diets was partially unwrapped and placed with each larva in an experimental group each day. A modification of this procedure was that equal amounts of beef liver and chicken burger were homogenized together in a blender, and 1-ml volumes wrapped and frozen as described.

In the first experiment, beef liver, calf liver, chicken burger, hamburger, pork, tunafish, and catfood were fed to each of 20 newly-eclosed *C. sycophanta* larvae. The second experiment was similar, except that only beef liver, calf liver, chicken burger, hamburger and pork were tested, and there were only 19 replicates for the calf liver and chicken diets. In the next experiment, beef liver, chicken burger, or a piece of both beef liver and chicken liver was placed with each of 10 larvae. In the next four experiments, beef + chicken burger was tested against larvae receiving gypsy moth larvae. In the first of these experiments, raw beef liver and chicken were fed as separate chunks to each larva (20 replicates each). In the second, the liver and chicken were fed to larvae as before, but they were first autoclaved before being wrapped in plastic and frozen (20 replicates each). In the third, homogenized raw beef liver + chicken was fed to 20 experimental larvae while 21 other larvae were fed gypsy moth pupae. In the last of these experiments, 20 larvae were fed gypsy moth pupae, 20 fed homogenized raw beef liver + chicken, and 21 fed raw beef liver + chicken in separate pieces.

As an indication of how successfully larvae could be reared on an artificial diet in groups, another experiment was conducted in which larvae were reared as described by Weseloh (1996). Forty to 50 neonates were placed in a 31 by 21 by 10 cm plastic crisper having about 3 cm of moist peat moss in the bottom. Larvae were either provided about 20 gypsy moth pupae daily, or an equivalent number of "jelly rolls" of homogenized beef liver + chicken placed on a 1-cm mesh hardware cloth that was placed over the peat moss. The "jelly rolls" were assembled by spreading homog-

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enized beef liver + chicken over a sheet of plastic wrap to a thickness of about 2 mm. The sheet was then rolled to a 1-cm diam. The rolls were frozen and chopped into 4-cm lengths that were stored frozen until needed. Six such crispers with gypsy moth pupae, and four with artificial diet, were set up in September 1995. Larval survival, elytra length, and pronotum width of each resulting adult were determined.

Data were not transformed because there was little variance heterogeneity as determined using Bartlett's test for homogeneity of variance (Wilkinson 1988). Length of time in the various stages, elytra length, and pronotum width were analyzed by analysis of variance, with diet type and sex being factors. Survival of beetles to adulthood was analyzed by contingency tables, using maximum likelihood chi-square to assess significance.

Results and Discussion

Sex was generally not a significant factor in the analyses of variance. Thus, except for the last experiment, results for sex will not be reported. Survival of beetles on diets varied greatly, with survival better in the second experiment than the first (Fig. 1).

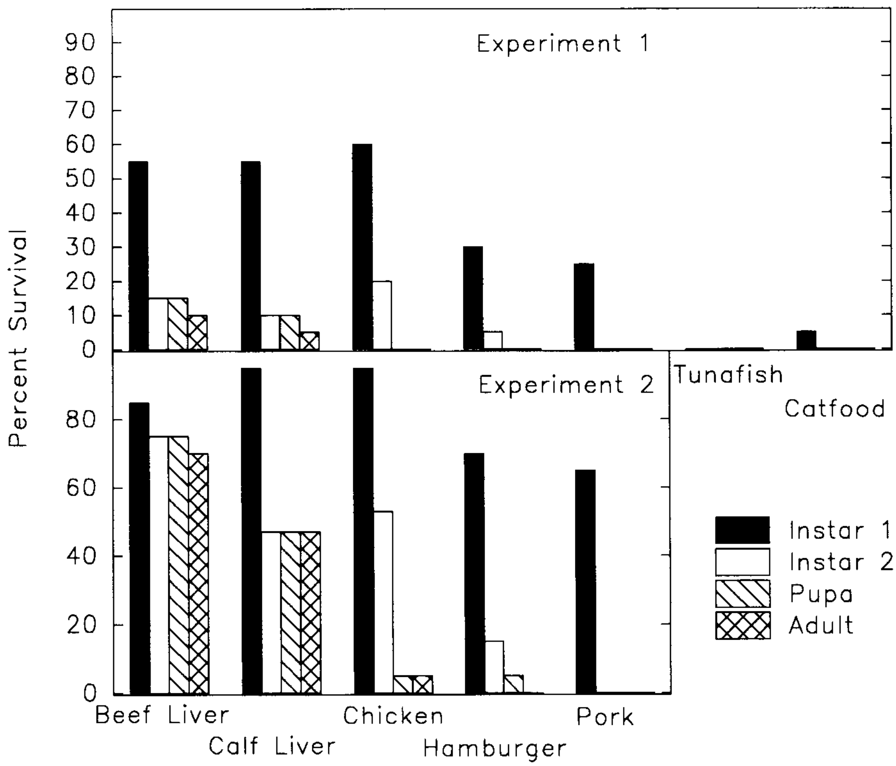

Fig. 1. Survival of *Calosoma sycophanta* larvae on various artificial diets, reared individually.

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Possibly the low survival rates in the first experiment were due to suboptimal rearing conditions. Clearly, raw beef liver and calf liver supported the greatest survival, with almost none of those fed tunafish or catfood surviving past the first stadium.

Based on the results of the first experiments, I felt it might be useful to combine beef liver and chicken. I hypothesized that these two diets are sufficiently different that the combination may supply nutrients lacking in one of them. This appeared to be the case. When raw beef liver was tested against chicken and the combination of beef liver + chicken (presented as separate chunks), chicken by itself was not suitable, but there were small differences in developmental times of larvae fed beef liver only or beef liver + chicken. However, the beetles fed both were generally larger than those fed beef liver only (Fig. 2). Contingency-table analysis comparing those fed beef liver to those fed beef liver + chicken showed no significant differences in survival (P = 0.369).

Results were similar whether or not the beef liver and chicken were in separate chunks or homogenized, so long as they were raw (Fig. 3A, C). Developmental times of instars 1, 2, and pupae were similar, and shorter for instar 3 for those fed raw, mixed diet as compared to those fed gypsy moth pupae. However, larvae fed gypsy moths consistently produced the largest adults, as measured by elytral length and

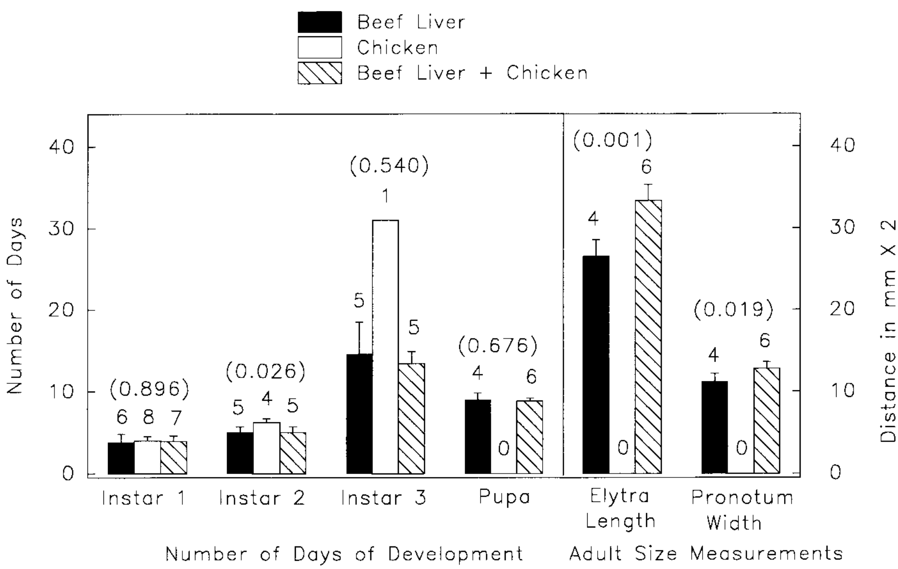

Fig. 2. Number of days in each instar, and elytra length (mm × 2) and pronotum width (mm × 2) of adults, of *Calosoma sycophanta* reared on beef liver, chicken meat (chicken burger) or both. Brackets indicate standard deviations, numbers immediately above each bar are the number of individuals for which the data were measured, and the fractions in parenthesis are the P-values for differences between diets in the analysis of variance. Data for the chicken diet were included in the analysis of variance only for instars 1 and 2 because too few fed chicken only survived beyond the second instar.

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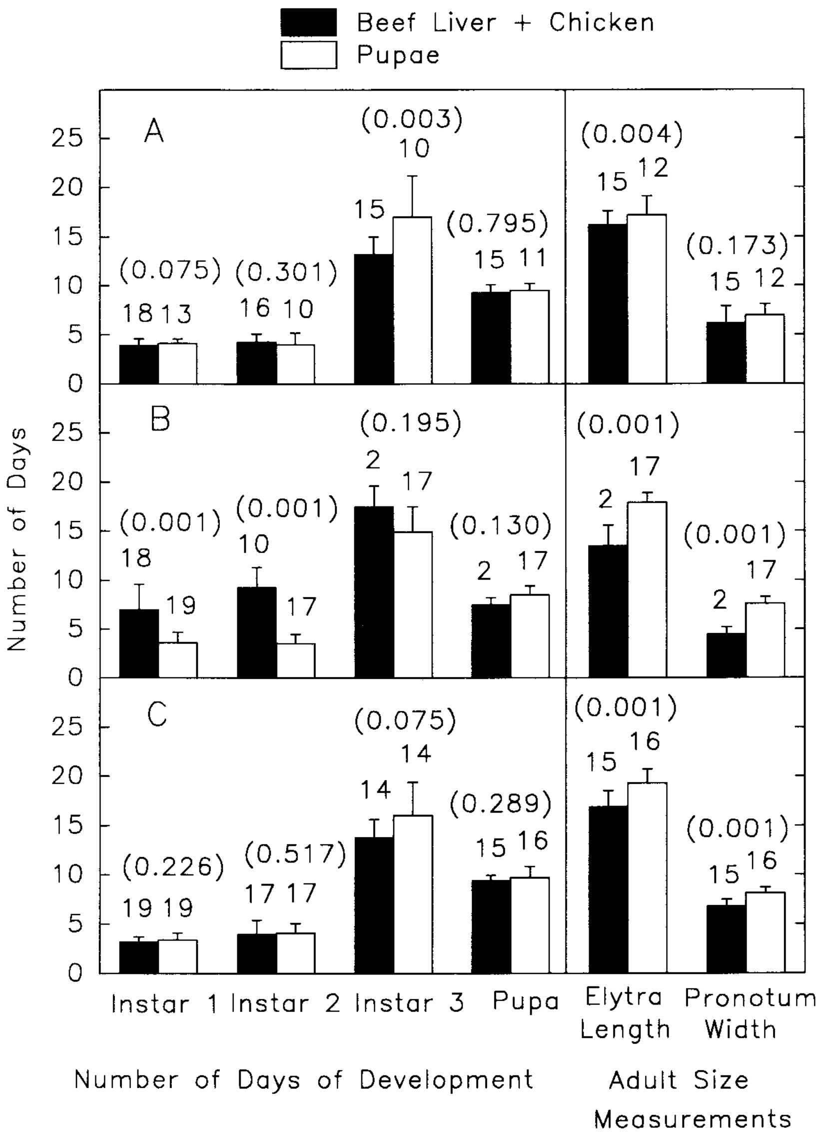

Fig. 3. Number of days in each instar, and elytra length and pronotum width of adults, of *Calosoma sycophanta* reared on gypsy moth pupae and (A) raw beef liver + chicken, presented as separate pieces, (B) autoclaved beef liver + chicken, presented as separate pieces, and (C) raw beef liver + chicken, homogenized. Brackets indicate standard deviations, numbers immediately above each bar are the number of individuals for which the data were measured, and the fractions in parenthesis are the *P*-values for differences between diets in the analysis of variance.

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pronotum width. Survival of beetles, as determined by contingency table analysis, was not significantly different between larvae fed raw, mixed diet or those fed pupae in these two experiments (P = 0.310 and 0.705, respectively). Autoclaved beef liver + chicken was an inferior diet for *C. sycophanta* larvae (Fig. 3B), resulting in poor survival compared with those fed gypsy moth pupae (P < 0.001).

An experiment in which larvae were fed raw, homogenized beef liver + chicken, separate pieces of raw beef liver + chicken, or gypsy moth pupae, provided similar results (Fig. 4). Developmental times of all stages were similar for all diets, and larvae receiving gypsy moths consistently produced the largest adults. Survival was the same for larvae fed any diet (P = 0.089).

Among larvae reared in groups, those fed pupae and reared in groups had greater survival (58.1%) than those fed homogenized beef liver and chicken (44.2%) (P = 0.004). Also, larvae fed gypsy moths produced the largest adults, with females typically being larger than males (Fig. 5).

Of the diets tested, a combination of raw, homogenized beef liver and chicken permits satisfactory development of *C. sycophanta* larvae. There appears to be a synergism between the nutritional value of beef liver and chicken, in that adult beetles were somewhat larger when both diets were provided than when only one was (Fig.

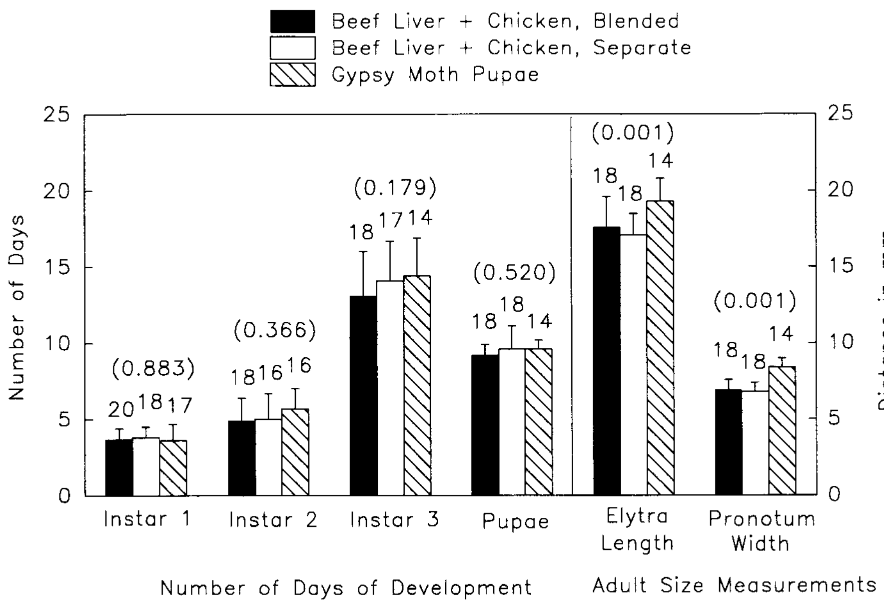

Fig. 4. Number of days in each instar, and elytra length and pronotum width of adults, of *Calosoma sycophanta* reared on gypsy moth pupae or homogenized raw beef liver + chicken, or raw beef liver + chicken presented as separate pieces. Brackets indicate standard deviations, numbers immediately above each bar are the number of individuals for which the data were measured, and the fractions in parenthesis are the P-values for differences between diets in the analysis of variance.

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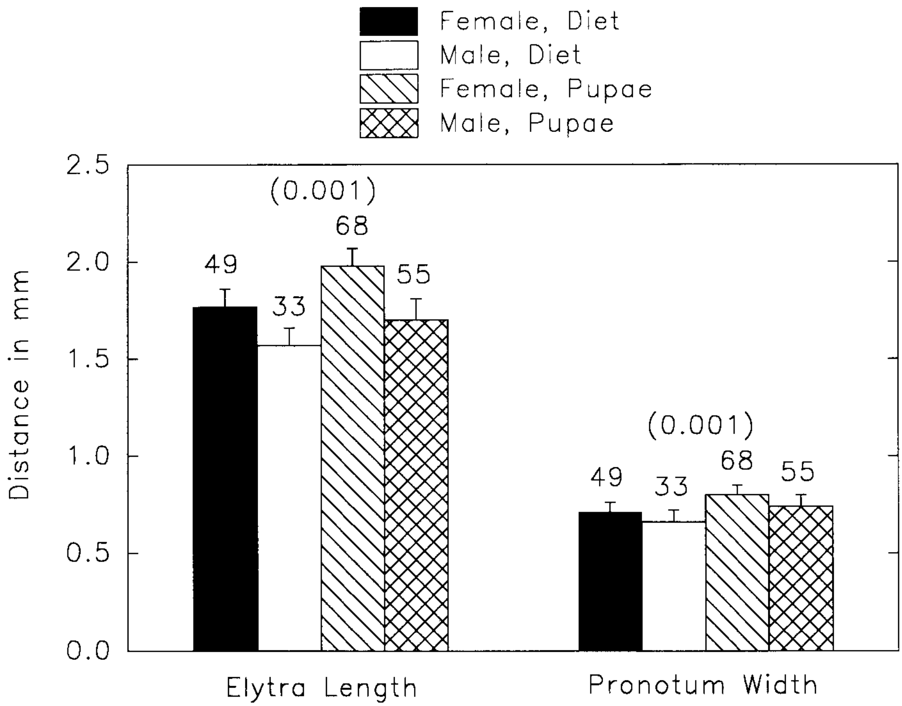

Fig. 5. Elytra length and pronotum width of *Calosoma sycophanta* males and females, the larvae of which were reared in groups on gypsy moth pupae or homogenized raw beef liver + chicken. Brackets indicate standard deviations, numbers immediately above each bar are the number of individuals for which the data were measured, and the fractions in parenthesis are the *P*-values for differences between diets in the analysis of variance.

2). Together, beef liver and chicken provide more complete nutrition than either one alone. Beetles reared individually on this artificial diet have similar developmental times as those fed gypsy moth pupae, except that diet-fed larvae tend to spend less time in the third stadium (Fig. 3). Diet-fed larvae are somewhat smaller than those fed gypsy moth larvae, and so perhaps diet-fed larvae complete feeding sooner or need less time after finishing eating to prepare for pupation.

The beef liver + chicken diet developed and tested here is not equivalent to gypsy moth pupae as food for *C. sycophanta* larvae. Not only are adult beetles smaller when larvae are fed diet, but when reared in groups, larvae also do not survive as well as those fed pupae. Adult beetles do feed on this diet, but their egg production is low and sporadic as compared to when they are fed gypsy moth larvae (pers. observ.). Research is in progress to develop a suitable adult diet. Despite these disadvantages, the size and survivability of larval beetles fed this mixture are close to what can be achieved using gypsy moth pupae as food. This convenient diet should be useful in efficiently rearing large numbers of this predator.

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