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LABORATORY MASS-REARING OF *CISSEPS FULVICOLLIS* (CTENUCHIDAE), WITH NOTES ON FERTILITY, FECUNDITY, AND BIOLOGY

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I. INTRODUCTION

In the course of an investigation into the genetics of certain pupal and imaginal characters in *Cisseps fulvicollis* (Hübner) in 1956 and 1960-62, it was found that this diurnal moth is an excellent species for continuous mass-rearing in the laboratory. Its foods (various species of grasses) are widely obtainable in the outdoor growing season, or easily produced in the laboratory. It mates readily and oviposits freely in confinement. The duration of the life cycle is fairly short and there is no diapause under laboratory conditions, so successive generations can be reared continuously indoors, even through the winter. The adults are not vigorous flyers, which makes handling and feeding more convenient than for many Lepidoptera.

In the following sections, the techniques used for mass-rearing of *C. fulvicollis* are described, followed by observations on its biology, which were made as an adjunct to the genetic investigations. Also included are comments on comparable published findings reported for a few other Lepidoptera, to put the *Cisseps* observations in some perspective. No attempt was made to search the literature thoroughly, and the papers referred to are undoubtedly only a few of those which exist.

II. TECHNIQUE

This project was conducted in a windowless laboratory room, at a constant temperature of 22°C., under a bank of "daylight" fluorescent lights automatically set on a 24-hour cycle with 14 hours of light alternating with 10 hours of dark.

Females were isolated in circular clear plastic (polystyrene) containers about 8 cm. in diameter by 4.5 cm. deep. They were daily moved to fresh dishes when ova were laid, if a daily count of ova was desired; otherwise, they were left in the same container for the duration of oviposition. Every second day each adult was fed from a small disc of synthetic cellulose sponge saturated with a mixture of 1 part honey to 2 parts water. For feeding, each oviposition container was inverted over a Syracuse dish holding the sponge; the moths normally started feeding voluntarily within a few seconds, but during the first 24 to 48 hours they seemed disinterested in feeding. They were left on the sponge for about 10 minutes, or less if they had discontinued feeding.

The widespread techniques of feeding Lepidoptera adults in the laboratory from saturated wicks or small containers of sugar solution left in the cages have been tried at various times on many species in this laboratory. However, the fermentation of the solution which takes place rapidly in a warm room has been found to be detrimental to optimum egg production. Perhaps some substance such as calcium propionate could be added to retard this process. Another drawback to that type of feeding apparatus is that many adult Lepidoptera will not find the food source or will not learn to feed regularly from it. When our sponge method is used, they will inevitably come into contact with the sugar solution and will usually start feeding immediately; if not, the technician can unroll the proboscis with the aid of a dissection needle, and this often stimulates the insect to feed. Fermentation is eliminated because the solution is freshly mixed each day and is left out for only a short time, and the sponge and dish are thoroughly rinsed and dried between uses. The sugar solution should not reach the top of the sponge; it is enough to have it near the top so that the insect can reach into the holes in the sponge with its proboscis but will not get its legs sticky. The sponge method is especially important in crucial crosses where the maximum quantity of eggs if needed. With a few exceptions for groups with non-feeding adults, such as Saturniidae, feeding is essential if the breeder wishes to get maximum fecundity in confinement. In a tropical ctenuchid, *Ceramidia butleri,* HARRISON (1959) got only 56 eggs from 14 wild females, but the low number may be due to failure to feed the females.

The female firmly glued the eggs to the plastic container, often in linear strings of several eggs, and there was no need for grass to be present as an oviposition stimulator. When eggs had been laid in a container, a small piece of damp paper towel was put in to provide moisture and was redampened when dry. When the larvae were about to hatch, a few blades of fresh grass were put into the container to prevent the starvation and dessication which easily occur in the brief absence of food and moisture and are especially dangerous to newly hatched larvae.

The newly hatched larvae often, but not always, ate the eggshell immediately after hatching. SHOREY, *et al.* (1962) observed in laboratory rearing that undisturbed *Trichoplusia ni* larvae always did, although experiments showed no survival advantage during the first four days after hatching for those larvae which were allowed to do so compared to those removed from the hatching cage immediately after hatching.

All the 1956 larvae and the first generation of the 1960-62 broods were fed on outdoor lawn grass, which was dug up in circles of sod, put in clay flower pots, and brought into the laboratory. The second generation in 1960 was fed on laboratory-raised *Poa* sp. and *Festuca* sp. In 1961, *Poa pratensis* (Kentucky Blue Grass), *Lolium multiflorum* (Common Rye – narrow bladed) and *Lolium perenne* (Perennial Rye), all grown from seed, were tested and were all found to be unsuitable as food plants; the larvae commenced feeding but did not survive. The winter generations were reared successfully on *Festuca rubra* (Creeping Red Fescue) grown in the laboratory; this grass grows from seed to the usable height of about 2 inches in 7 days. *Stenotaphrum secundatum* (St. Augustine Grass) was an acceptable foodplant, grown from cuttings from Florida stocks; its wide blades and slowness to wilt when cut made it an ideal food, but it grew so slowly in the laboratory that it could not be used exclusively.

The grasses were grown in vermiculite in polyethylene boxes with holes drilled in the bottom. They were fed from below by a nutrient solution twice a day in an automatic hydroponics tank-table.

During the first two instars of the larval stage, it was most convenient to cut the grass and put small amounts of it in the larval rearing dishes. The bottom and sides of the dishes were lined with paper toweling; this could be changed easily when necessary, and it kept the containers relatively free of water droplets, which are dangerous to small hairy larvae. Four small holes were drilled in the top of each container to facilitate the movement of air. Before the tops were perforated, it was found that the larvae would become anaesthetized, apparently from accumulated carbon dioxide, with high mortality resulting.

A free-standing shelf of copper wire screening, consisting of a circle about ⅔ the diameter of the dish and supported by a strip of bent screen, was used in each larval rearing dish. Each day or two, when the dish was cleaned, the shelf was lifted out, with most of the larvae clinging to it. The paper at the bottom, containing the feces and old grass blades, was discarded and new paper put in. The side-lining paper could be returned to the dish with the larvae still clinging to it. Then the grass on the shelf, with some larvae on it, was put in the bottom and new grass placed on the shelf. The larvae usually climbed quickly up to

the new grass, and thus did not need to be handled individually when the dish was next cleaned. Handling leads to increased mortality, particularly of those larvae in a pre-molt phase.

To defer growth, or if foodplant temporarily runs short, larvae in the middle instars can be stored for several weeks in a cold chamber. A constant-temperature laboratory room, kept at 14-16°C., was used successfully, but the much lower temperature of a refrigerator at common household setting was fatal to the larvae. In the cold room they were kept in the regular rearing containers, partly filled with slightly damp (not wet) sphagnum moss in which they burrowed.

When the larvae were in the last instars they were transferred to gallon polystyrene jars with screen tops. Grass was supplied either by being grown in the container, or by putting two of the cubical polyethylene dishes containing growing grass in each gallon container. In the former case, if few enough larvae were put into it and the container left on the hydroponics table, the grass growth kept pace with larval consumption. In the latter case, the two cubical dishes had to be removed every two days and the larvae transferred to new dishes of grass.

There was very little evidence of disease in the course of these experiments. Ordinary clean laboratory procedures were used, but no sterilization of equipment was necessary.

Pupation occurred in loose cocoons spun on the grass blades or on any surface within the container. In the case of the "cocoonless" individuals (those having a supposedly genetic tendency to pupate without spinning a cocoon), the pupae were lying free on the bottom. Pupae were put into small glass shell vials, closed with a loose wad of cotton, and permitted to hatch there.

The adults were confined in pairs in polystyrene containers under the "daylight fluorescent lights. If no ova were laid within a week, the first male was removed and a second male introduced. In some cases a male whose mate had produced fertile ova was paired with a second female.

The sexes are not difficult to distinguish in the adult moths when pairs are being selected for isolation. The best criteria are:

1) The pectinations of the male antennae are long (their length being about twice the width of the flagellum) and narrow and almost parallel-sided; the corresponding rami of the female antennae are much shorter (about the same length as the width of the flagellum) and rounded, somewhat oval or club-shaped.

2) In the male the frenulum consists of one stout spur, and in the female there are two, slenderer spurs; in both sexes these are long (about 0.3 the length of the hind wing, from which the frenulum arises near the base), slender, shiny, and copper-colored. The receptaculum for the

frenulum is on the under side of the forewing and is sexually very different, in the male being a single thumb-like hook arising from the anterior portion of the forewing in the male and in the female being a tuft of many stiff, hair-like bristles arising from the posterior portion of the forewing. These distinctions are easier to use than differences in the abdominal apex.

In the pupal stage, or on the empty pupal shell after the moth has emerged, the sexes can be distinguished by the position of the genital aperture. In the males, it is located on the 9th sternum, close to the anal aperture; in the females it is on the 8th sternum, about twice as far from the anal aperture as it is in the males.

III. Mating Behavior

In most of the cases in which copulation was observed, its duration was 2 to 5 hours. The original 1956 wild pair, which had been captured *in copulo,* remained together for several hours despite being gently transferred to a container and taken in an automobile to the laboratory. The longest observed mating was of a pair which had been inactive for three hours (7-10 A.M.) under distant fluorescent ceiling lights and some light at a very oblique angle from "daylight" fluorescent lights. They paired immediately at 10 A.M. when put under the direct light of a 150 watt desk lamp, and they remained paired for at least 12 hours, 20 minutes but had separated by 8:55 the following morning.

Both males and females of 12 observed pairs varied in age from 1 to 15 days at time of the first observed copulation. None were seen to mate before 24 hours after eclosion.

Some pairs were seen to copulate twice, the second time following the first by as little as an hour or as long as 8 days. One male mated with 3 females, when he was 6, 9 or 10, and 14 days old; the first of these was unsuccessful, with none of the 152 eggs laid showing signs of any development. One pair which had been confined together for 8 days and had already produced fertile eggs, was found in subsequent copulation which lasted at least 5 hours, 45 minutes (this was the female which had a late, second peak in number of eggs laid; see Table I). Another pair which had been together for ten days, with no eggs laid, was then observed *in copulo* for at least 3 hours, 13 minutes. Two pairs which had mated once with only one to two ova produced were subsequently found *in copulo,* but either none or only a few further eggs were laid. However, in other cases the females accepted the second pairing even though fertilized eggs had been laid right up to the day of re-pairing.

Mating always commenced in the forenoon, soon after the lights in the windowless control room went on. On one occasion, many pairs were

put in a light-proof dark chamber at 3:45 P.M. and then removed to bright light at 2:05 P.M. the following day. No pairings were made during 2½ hours of subsequent observation.

The pairs usually rested on the bottom surface of the container when *in copulo,* but if they were on the side of the container, they were vertically oriented, the male usually head downward.

DISCUSSION

Duration of copulation was not shown to have an effect on the success of fertilization. The longest duration (at least 12 hrs.) resulted in fertilization. The exact duration of the shortest was not observed, but among those with the least time, there were many fertilizations. This may be contrasted with results of 7 *Papilio xuthus* pairings in which the 3 of very short or very long duration produced no fertile eggs (Remington, 1960). It was observed in these *Papilio* matings that "long duration is usually caused by abnormal initial coupling, in which event insemination is not effected and disengagement is difficult". The long *Cisseps* mating noted here was disengaged without artificial help, although in some other cases both members of a pair remained together and died; if artificial separation was made, it resulted in such damage to the individuals that they lived only a short time and the females were unable to oviposit.

The duration of copulation has been found to vary with the temperature in *Pieris brassicae* (David & Gardiner, 1961). Temperatures of 20°, 25°, and 30°C. were used, and copulation lasted longer at the lower temperatures. In a different experiment, five pairs of *Trichoplusia ni* at 27°C. remained *in copulo* an average of 33 minutes, but the period varied from 19 to 44 minutes (Shorey, *et al.,* 1962).

Pieris brassicae females did not usually pair again for 5 or more days after the first mating, although males often mated several times in one day. Of 20 ♀ ♀, 2 mated twice the first day of the test (when they were 2-3 days old) and one of the 2 again on the second day. By the 10th day, 18 of the 20 had mated twice (one mated 3 times, 2 mated 4 times). In another group of *brassicae* 5♂♂ mated 1 to 4 times in 5 hours, and some of the 15 ♀ ♀ twice in the 5 hours (David & Gardiner, 1961).

94 ♀ ♀ of *Trichoplusia ni* confined continuously with a large number of males mated successfully a mean of 2.0 times (dissection showed 0 to 6 spermatophores) and the males more, since there were fewer males than females in the experimental cage.

The age of adults when ready to mate appears to be about the same in *Cisseps, Pieris,* and *Trichoplusia,* in the observations noted here. No

matings were observed before 24 hours of age in *Cisseps*. In *Trichoplusia ni* no matings were observed until the second night after emergence (this species is nocturnal). *Pieris brassicae* adults did not mate readily until a "day or two" after emergence, and it was unusual for either sex to mate when less than 18 hours old. *Pieris* adults stored at 12.5°C. and 60% humidity until 7-10 days old mated readily when put in 29° rooms, and all eggs were fertile. However, after storage of 19-20 days most ♂♂ died; the ♀♀ mated with 7-10 day old ♂♂ but fertility was lowered.

Although *Pieris brassicae* and *Cisseps fulvicollis* are both diurnal insects, *Cisseps* was found to commence mating (in the laboratory) soon after the light went on, whereas *Pieris* was said to favor no particular time of day for mating as long as the temperature and light conditions were satisfactory.

IV. Fecundity

The data on fecundity are based on 12 females, laboratory-reared siblings from one pair of wild parents.

Oviposition very rarely began within 24 hours after copulation, or as long as 72 hours after; most commonly it began between 36 and 48 hours after copulation. The females were from 2 to 16 days old when oviposition commenced; each had been confined with a male within a few hours after her eclosion from the pupal shell. There was a fairly even distribution of ages between the two extremes. One female (1-f) continued laying fertile eggs 15 days after her last contact with a male, and two other females for 8 and 9 days, respectively.

The greatest percentage of ova (22.8%) were laid by this group on the 5th and 6th day after oviposition began, the number thereafter declining steadily, as shown in Table I and Figure 1. However, two females (1-e and 1-f) laid their greatest number the 1st and 2nd day of oviposition, ♀ 1-g and ♀ 1-l laid their largest number the 3rd and 4th days, ♀ 1-j had a peak on the 7th and 8th days, and ♀ 1-d on the 9th and 10th days. For the last-named ♀, copulation had been observed on the day before and this was at least the second of her pairings, for she had already laid fertile ova; after this peak she went on ovipositing for 10 more days before her death.

There was also great variation in total number of eggs laid. Each of the 12 females laid a total of from 104 to 299 ova in an ovipositing period of 8 to 20 days.

Discussion

The findings with *Cisseps* that the greatest percentage of eggs were laid on the 5th and 6th days of oviposition differs from those with a

Table I. DISTRIBUTION OF EGG-LAYING OF 12 SIB-MATED, LABORATORY-REARED ♀♀ OF *C. FULVICOLLIS*.

Mother	Days after start of oviposition									
	1 & 2	3 & 4	5 & 6	7 & 8	9 & 10	11 & 12	13 & 14	15 & 16	17 & 18	19 & 20
♀ 1-a	67	8	82	24	8*	–	–	–	–	–
♀ 1-b†	2	4	53	18	10	26	22	17*	–	–
♀ 1-c	6	24	52	33	40	44	4	2*	–	–
♀ 1-d	41	0	29	37	95	21	11	11	16	6*
♀ 1-e	86	54	25	0*	–	–	–	–	–	–
♀ 1-f	43	14	0	0	0	17	26	4*	–	–
♀ 1-g	51	68	22	18	43	38	10	1*	–	–
♀ 1-h	24	53	62	0	48*	–	–	–	–	–
♀ 1-i	8	42	92	51	5	46	39	16	0*	–
♀ 1-j	38	11	15	74	40	36	15*	–	–	–
♀ 1-k	69	0	89	53*	–	–	–	–	–	–
♀ 1-l	16	43	39	47	32	22*	–	–	–	–
Total	451	321	560	355	321	250	127	51	16	6
%	18.3	13.1	22.8	14.4	13.1	10.2	5.2	2.1	.7	.2

† None of her eggs developed, although copulation had occurred.
* Found dead here, in routine alternate-day inspection.

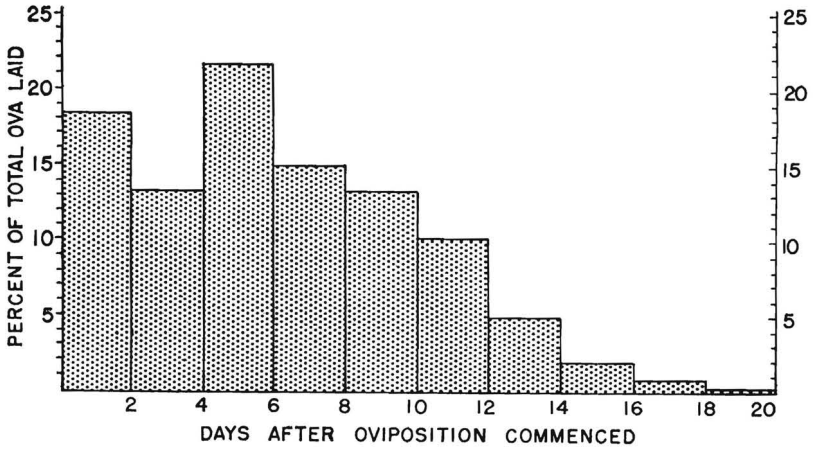

Fig. 1. Percentage of total ova laid by 12 ♀ ♀ in successive two-day periods (see Table I for individual performances).

phycitid moth, *Anagasta* (=*Ephestia auctt.*) *kühniella,* in that the latter laid the greatest number the first two days, then fewer each successive day (Norris, 1936). The *Cisseps* oviposition activity is similar to that of 99 females of *Trichoplusia ni* in which the most eggs (32%) were laid on the 5th and 6th days after commencement of oviposition (McEwen & Hervey, 1960). [Another group of *Trichoplusia* had low (23%) hatchability, indicating mating difficulties, and these data are not reviewed here.] The *Trichoplusia* females laid for only 12 days, while *Cisseps* laid for 20 days. David (1957) found that *Pieris brassicae* females start laying 2 to 3 days after eclosion and reach a maximum in 2 to 3 days more.

V. Fertility and Hatchability

Of 426 ova laid in the laboratory by 2 wild females (Table II), 99% showed the signs of fertilization (early stages of differentiation in the embryo). Among 1727 ova laid by 14 sibling-mated laboratory-reared females the percentage of ova showing signs of fertilization was almost as large: 97.1%. It may be observed from Table III that there was no decline in fertility with age; note the high hatchability (and therefore fertility) even late in the oviposition period, for 3 of the 4 females (the 4th had sharp fluctuations, which are not directly correlated with age).

Table II shows a great difference in hatchability of the eggs between that of the wild, presumably outcrossed, females (98.1%) and that of the inbred females (59%). But Table III again shows that there was

Table II. FERTILITY AND HATCHABILITY OF OVA.

A. Ova from Wild Females.

Egg layer	Number laid	Number hatched	Number unhatched	
			showed some development	showed no development
♀21	233	232	1	–
♀22	193	186	3	4
Total	426	418 (98.1%)	4 (0.9%)	4 (0.9%)

B. Ova from Sib-mated Females.

♀1-a	67	67	–	–
♀1-d	148	58	79	11
♀1-f	47	0	16	31
♀1-i	8	2	6	–
♀1-l	98	8	90	–
♀21-a	103	99	4	–
♀21-e	111	80	31	–
♀21-j	179	76	97	6
♀21-k	210	117	92	1
♀21-m	235	208	27	–
♀21-u	2	0	1	1
♀22-d	193	133	60	–
♀22-e	168	76	92	–
♀22-f	158	99	58	1
Total	1727	1023 (59.2%)	653 (37.9%)	51 (3.0%)

Table III. PERCENTAGE OF HATCH FROM OVA LAID ON SUCCESSIVE DAYS.

A. Wild Females.

Egg-layer	Date	No. laid	No. hatched	% hatched
♀21	Oct. 4-5	89	89	100.0
	Oct. 5-6	35	34	97.1
	Oct. 7-10	64	64	100.0
	Oct. 10-11	9	9	100.0
	Oct. 15-18	36	36	100.0
♀22	Oct. 4-5	34	30	88.2
	Oct. 5-6	40	40	100.0
	Oct. 6-7	13	13	100.0
	Oct. 7-10	106	103	97.2

B. Sib-mated Females Reared in Laboratory.

♀21-m	Nov. 17-18	87	80	92.0
	Nov. 19-20	29	28	96.6
	Nov. 20-22	43	36	83.7
	Nov. 22-23	15	10	66.7
	Nov. 23-27	51	48	94.1
	Nov. 28-30	11	6	54.6
♀1-d	Nov. 12-13	31	12	38.7
	Nov. 19-20	33	6	18.2
	Nov. 21	62	38	61.3
	Nov. 22-27	43	11	25.6
	Nov. 29	20	2	10.0

no clear decline of hatchability due to the age of the mother or to the number of eggs she had laid; high percentages of hatch were maintained throughout the period of the 2 wild females and fairly high even in the middle and late stages for the 2 inbred females, although the hatch for the latter was lower and more uneven.

DISCUSSION

The high fertility of *Cisseps* eggs (97-99%) is paralleled in the fertility of eggs of wild *Colias philodice* and *eurytheme* (Ae, 1958); of 762 eggs laid by 16 females, 99% were fertile. AE also found (1961) that of 288 eggs laid by 3 wild females of *Papilio protenor* 99.3% were fertile; 98.2% of them hatched. The low (11.8%) fertility and hatchability of the 76 eggs of one wild *Papilio helenus* female was attributed to a shortage of spermatozoa, since the female was old when collected.

The eggs of *Trichoplusia ni* were found to be 80% hatchable in the laboratory at 14°C., and to decrease in hatchability to 70% at 32° (Shorey, *et al.*, 1962). In a different investigation on the same insect (McEwen & Hervey, 1960) 3 colonies at the same temperature (23-25°C.) varied widely in hatchability:

1st colony – 61 ♀♀ (15,793 eggs laid) – 40.1% hatched
2nd colony – 34 ♀♀ (6,731 eggs laid) – 23.0% hatched
3rd colony – 38 ♀♀ (13,337 eggs laid) – 73.4% hatched

The reasons for the variation could not be determined, but "eggs which failed to hatch showed no evidence of embryonic development and collapsed 2-3 days after deposition". This may have been due to non-fertilization.

NORRIS (1936) found that the proportion of eggs of *Anagasta kühniella* hatching from one sibling pair in each of 12 generations varied from 63 to 93%.

REMINGTON (1959) observed that it is "usual for *Papilio* females to have decreasing fertility in the course of egg-laying, regardless of the father."

VI. DURATION OF STAGES

The duration of the egg stage appeared to be 5 to 6 days. Twenty-one eggs (from ♀ 21-m) which were laid during a 10 minute period on 18 November were segregated and external changes during development at the controlled temperature were recorded as follows. This female had started ovipositing the day before and continued for 12 days thereafter; all eggs were fertile.

Age in hours	*Development*
24	Opaque white, no differentiation
48	No change
71	A faint transparency developing around lower edge
95	A cluster of minute brown spots at top; definite differentiation between zones of paler and deeper yellow
120	Larvae appear fully developed, visible through transparent shell
124	No change
125	First 2 larvae hatched
125.5	1 more larva hatched
126	3 more larvae hatched
126.75	4 more larvae hatched
127	6 more larvae hatched
128	1 more larva hatched
129	2 more larvae hatched
130½–140	Last 2 hatched during this period

Another group of timed eggs hatched later than 122 hours and before 144 hours. Out of a third group (38 eggs), 30 larvae hatched in about 120 hours, 1 between 120 and 144 hours, and 7 between 144 and 168 hours.

In a group of 9 timed larvae, there were 4 instars (varying in total duration from 17-21 days) before successful pupation for 7 of the individuals (5♂♂, 2♀♀). The 2 others had 6 instars and then died.

The pupal stage normally lasted 7-11 days. There was a sexual difference in developmental time. Of 22 larvae whose pupal stage began synchronously and which were reared together, eclosion was as follows:

	♂♂	♀♀
1st day	7	3
2nd day	4	2
3rd day	0	3
4th day	1	1
5th day	0	1

Thus, 11 of the 12 ♂♂ had emerged in the first two days and only 5 of the 10 ♀♀.

There was also a sexual difference in the length of the imaginal stage. For 12 females this stage varied from 12-30 days, with half of them living 22-30 days. For 8 males the duration varied from 3-23 days, with half of them living 18-22 days.

In summary, at the constant temperature of 22° C. and a 14-hour photoperiod, the normal life cycle in days was as follows: egg 5-6, larva 17-21, pupa 7-11. The minimum observed development from egg to adult was 29 days; the maximum (abnormal) 62 days.

In outdoor conditions *C. fulvicollis* is multivoltine and probably hibernates in the larval stage (Dyar, 1901). There was no diapause under laboratory conditions. As many as 5 successive generations were reared through the winter, and this could have continued indefinitely.

DISCUSSION

DYAR (1901) reported that some *C. fulvicollis* from New York City which he reared hatched from eggs about Sept. 15 and became imagos on Oct. 19 (a total of 39 days if the eggs took 5 days to hatch). COMSTOCK (1937) recorded a pupal duration averaging 6 days for individuals of *C. fulvicollis* collected in the Sierras of California. None of the pupae in the present experiments took as few as 6 days, although a few required only 7 days. Perhaps there is a genetic difference in developmental period between these and California populations, but COMSTOCK's pupae may have been kept at a higher temperature. Developmental time is under genetic, as well as environmental, control, and it can be presumed that in some regions natural selection favors genes for faster developmental rate than in other regions (see, *e.g.*, Dawson & Lerner, 1962).

The large observed variability of total developmental time in the present experiments has also been reported for other Lepidoptera. *Anagasta kühniella* varied from 88-137 days at "room temperature" over 12 generations (Norris, 1936). ATWAL (1955) reported that temperature, photoperiod, and quality and age of foodplant influenced the length of life cycle of *Plutella maculipennis* Curtis. While temperature and photoperiod in the *Cisseps fulvicollis* experiments were uniform, the varying quality and age of the grass brought in from outdoors may have influenced those broods whose life cycle was studied. LONG (1953) found that crowding of lepidopterous larvae increased the rate of development and the simultaneity of pupation, and decreased the number of instars. In the *Cisseps* experiment, the individuals which were closely observed during the larval and pupal stages were kept in solitary or uncrowded conditions, so that factor was negligible. *Trichoplusia ni* larvae had from 5 to 7 instars depending on conditions (Shorey, *et al.*, 1962).

The sexual difference in developmental time has also been observed in other Lepidoptera, such as *Plutella maculipennis* in which females developed more slowly than males, irrespective of temperature and food (Atwal, 1955). There is a widespread presumption that this is generally true for Lepidoptera, although *Trichoplusia ni* in the experiments of SHOREY, ANDRES, and HALE showed no sexual difference in the length of the larval stage on four kinds of food and at four different temperatures. In the pupal stage the females developed 4-6% *faster* than males when the larvae had been reared on beans, but not on cabbage.

Table IV. SEX-RATIO OF REARED ADULTS.

Brood	♂♂	♀♀
1st generation, 1956	11	14
2nd generation, 1956	19	10
1st generation, 1960	94	91
2nd generation, 1960	1	2
1st generation, 1961	15	21
2nd generation, 1961	78	53
3rd generation, 1961	149	128
4th generation, 1961	88	83
5th generation, 1961	74	47
Totals	529	449

VII. Sex-ratio

The sex-ratio of 978 reared adults was found to be 1.00 : 0.85 (see Table IV). In the five 1961 broods 45 others were in too poor a condition for accurate sexing, or were missing in both pupal shell and adult at the time of the sex-count.

The sex-ratio of 323 reared adults of *Trichoplusia ni* was 1:00 : 1.17, a predominance of females (Shorey, *et al.*, 1962).

Summary

1. The techniques used in the mass-rearing in the laboratory of successive generations of *Cisseps fulvicollis* are described.

2. Copulation was observed to continue for a period of 2 to 5 hours. Both males and females may copulate at least twice, the second pairing following the first by as little as an hour or as long as 8 days. Fertile ova may be laid before and/or after the second mating. Oviposition usually started within 48 hours after copulation and continued for 8 to 20 days totalling about 100-300 ova from each female. The greatest number of ova were laid on the 5th and 6th day after commencement. Females continued to lay fertile eggs 15 days or more after isolation from males.

3. 99% of the 426 ova from two wild females were fertile. 97.1% of the 1727 ova from sibling-mated laboratory-reared females were fertile.

Hatchability of the first group was 98.1%; of the second, 59.3%. Fertility and hatchability of ova laid on different days by any one female did not show a decline with age or number of eggs laid by the mother.

4. The egg stage usually lasted 5-6 days; siblings hatching from eggs laid almost simultaneously had different rates of embryonic development. Larval duration was usually 17-21 days, pupal duration 7-11 days. Total cycle from new egg to adult eclosion varied from 29 to (rarely) 62 days, at 22° C. and 14 hours photoperiod. Males developed faster than females. There was no diapause under laboratory conditions.

5. Of 978 reared adults, 529 were males and 449 females, a sex-ratio of 1.00 : 0.85.

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