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This investigation is concerned with the determination of the temperature-effective period (T. E. P.) for crossvein development at physiological temperatures using three isogenic crossveinless-like (cvl) lines of Drosophila melanogaster.

The method used to determine T. E. P. relies on temperature differences in phenotypes and on a program of transfers from one temperature to another at regular intervals during development.

The T. E. P. in each cvl line occurs in the interval between one-eighth and one-quarter of pupal development. The onset of T. E. P. in each cvl line is fairly well determined. The end points are not obtained in some cases and are ambiguous in others. Therefore, the durations of the T. E. P. 's cannot be stated. One of the complications in defining T. E. P. is the "switching effect", an effect on the phenotype due to transfer from one temperature to another.

Though temperature, genotypic and sex differences in T. E. P. are evident in some cases, theoretical interpretation of their significances are limited by the several complications.

TEMPERATURE-EFFECTIVE PERIODS DURING CROSSVEIN DEVELOPMENT IN CROSSVEINLESS-LIKE STRAINS OF *DROSOPHILA MELANOGASTER*

by

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TEMPERATURE-EFFECTIVE PERIODS DURING CROSSVEIN DEVELOPMENT IN CROSSVEINLESS-LIKE STRAINS OF DROSOPHILA MELANOGASTER

INTRODUCTION

Review of Literature

The induction of disturbances in the formation of the posterior crossvein by heat shocks during pupal development has been demonstrated in wild-type strians of different Drosophila species (Milkman 1962). It was found that crossveinless-like (cvl) strains of Drosophila melanogaster are especially susceptible to the induction of the crossveinless phenocopy (Mohler 1965a, Thompson 1966). Mohler (1965a) showed in a cvl line that the genetic basis of the phenocopy response is probably the same as that of the spontaneous phenotype. A reasonable working hypothesis is, therefore, to assume that the developmental processes in the formation of the crossvein at temperatures within and above physiological range are governed by the same mechanisms, and can be studied as a whole. The genetic make-up of cvl strains has been investigated by Mohler (1965b) and the two cvl systems he obtained were referred to as "major-gene-modifiers" polygenic systems.

Temperature responses in crossveinless phenocopy studies have been described in detail by Milkman (1961, 1962, 1963), Mohler

(1965a) and Thompson (1966). Mohler showed that the response to heat shocks in a cvl line not only resulted in an increase in penetrance and expressivity, but also in a qualitative change in the crossveinless phenocopy. Similar findings were reported by Thompson (1966) in two other cvl lines. Mohler suggested that there may be two opposing responses differentially expressed. The quantitative responses to heat shocks were found to vary with age (Mohler 1965a), and the period in development during which heat shocks are effective in the induction of the crossveinless phenocopy has been determined by the three workers cited above. Milkman (1962) found two periods of sensitivity in the wild-type strain, Oregon-R, with a major one at 24 to 25 hours and a smaller one at 18 to 19 hours of age in pupae raised at 23° C. In cvl lines, only one sensitive period was found within the same developmental range covering the two sensitive periods in Oregon-R (Mohler 1965a, Thompson 1966). In hybrid lines between Mohler's cvl strains and Oregon-R and Milkman's cvl strains and Oregon-R, two sensitive periods within the same developmental range were found, but different from that of Oregon-R, the major period of sensitivity is at 18 to 19 hours and the smaller one at 24 to 25 hours (Milkman 1961, Mohler 1965a). Responses in these hybrid lines were intermediate between these of the cvl lines and those of Oregon-R.

The thermal history of the pupae before heat shocks were

administered is an influencing factor in the response to phenocopy induction (Milkman 1962b, 1963). Temperature adaptation by exposing the pupae to temperatures within and above the physiological range has a protective effect against induction of crossvein defects (Milkman 1962b). Thompson (1966) found that heat shocks administered at certain times before the onset of the temperature-sensitive period can completely restore the posterior crossvein in two cvl lines.

The mechanisms underlining the temperature effects in crossveinless phenocopy studies are still not fully understood. Milkman (1962, 1963) proposed a model in which he postulated a protein essential for crossvein formation and that heat shocks destroy its function by changing its tertiary structure. This single model, however, does not provide an explanation for Thompson's finding that the complete crossvein can be restored in cvl flies.

Statement of the Problem

Phenocopy studies dealt with the effect of high temperatures, temperatures well above physiological range. Such studies strongly suggest that there is a period in the Drosophila pupae during which physiological temperatures affect crossvein development.

This thesis is an attempt to define and compare the temperature-effective periods (T. E. P. 's) of three cvl lines at 18°, 23° and 28° C.

The specific questions asked were: (1) What period during pupal development does temperature affect the expression of crossvein interruptions? (2) Is the time of the T. E. P.'s the same in different cvl lines and at different temperatures relative to the time of development as a whole?

MATERIALS AND METHODS

Three isogenic lines, "cvl-6b-hiX, 6b-hiII, 6b-hiIII", "cvl-6b-loX, OR-II, III(Ives)", and "cvl-5-hiX, 5-hiII, 5-hiIII (Curly float)", hereafter referred to as 6-hi, 6-lo, and 5-hi Cy^+ (with 5-hi Cy) respectively, were used. The cvl phenotypes of 6-hi and of 6-lo depend upon a major gene on the X chromosome and polygenic modifiers on the autosomes, and that of 5-hi depend upon a major gene on the third chromosome and polygenic modifiers on the X and second chromosomes (Mohler 1965b). These lines, obtained from Mohler, were constructed by methods described by him (1965a). For the 6-hi and 5-hi lines, constructed by Mohler, modifiers giving high phenotypic expression (high modifiers) were selected and made isogenic. For the 6-lo line, constructed by Thompson, low modifiers from the cvl-6b strain were made isogenic on the X chromosome and low modifiers from the inbred wild-type strain Oregon-R-Ives, were made isogenic on the autosomes. The 5-hi stock also carries the balancer chromosome Curly. It was put into the stock to improve fertility, which is low in the 5-hi Cy^+ line.

The following manner of handling cultures and of treating the pupae were strictly observed. Thirty to forty flies (about half males and half females) were placed in each bottle and subcultured every three or four days. After three consecutive subculturings, new flies

from stock sources were used. The eggs and larvae grew at 25° C in the standard cornmeal-agar-molasses-propionic acid medium, and were supplied with a thick suspension of fresh yeast on the fourth or fifth day. White prepupae, about 50, were picked from these cultures within a period of ten minutes and placed onto the walls of shell vials plugged with moistened cotton. Whiteness of the prepupae insures that they were collected within an hour after puparium formation (before noticeable tanning took place). All pupae were raised at the desired temperature in precision water baths.

A temperature coefficient for development was defined in order to compare the T. E. P. 's at various temperatures. Two different end points were used to determine the temperature coefficient: (1) Time of ecolosion to provide a rate of overall pupal development, and (2) the time of longitudinal wing vein development in the pupa to provide a rate of early pupal development.

The first end point was obtained as follows. Six to nine samples of white prepupae were collected hourly so that the time difference between consecutive samples was one hour plus or minus ten minutes. These samples were raised together at the required temperature. The number of flies emerged in each sample were counted at a time when nearly all the pupae in the oldest sample have emerged and few or no flies appeared in the youngest one. The number of flies that emerged in each sample was expressed as a percent of the

total number that emerged in that sample. These percentages were plotted against time on probability paper and fitted to a straight line by eye and the 50 percent point was taken as an estimate of the mean emergence time to the nearest quarter of an hour. The developmental time for the pupae of 6-hi, 6-lo at 18°, 23° and 28° C and 5-hi Cy^{+}, 5-hi Cy at 23° and 28° C were determined in such a manner.

The second end point was obtained only for 6-hi. Samples of pupae age 18 to 23 hours at 28° C and 25 to 31 hours at 23° C, were fixed by placing the vials in boiling water for ten seconds. Wing mounts from these pupae were prepared in a fashion described by Mohler and Swedberg (1964). One wing, taken at random from each pupae, was mounted. These mounts were examined under the microscope (50x) and the morphology of the logitudinal veins was compared with a standard developmental grade that is defined by the photographic print of a wing mount from a 24 hour old pupae grown at 25° C. This point is reproduced in Figure 20. Ten mounts of each age at each of the two temperatures were scored as prior to (-1), indistinguishable from (0), and beyond (+1) the standard developmental grade. The ages in hours of the series of ten wing mounts at each temperature bearing the closest resemblance to the standard were used to calculate the temperature coefficient (Q_5).

Preliminary determination of the T. E. P. in each cvl line was carried out by making transfers from one temperature to another

at times equivalent to one-half, one-quarter and one-eighth or pupal development. As shown in the result section, T. E. P. 's of all three lines were found to lie within the period between one-quarter and one-eighth of pupal development. To determine T. E. P. 's in a more precise fashion, transfers were made at hourly intervals within this period defined by the preliminary experiments.

The method of measuring cvl phenotypes was described by Mohler (1965a. b). A sample of flies is scored according to penetrace, expressivity and specificity. Breaks, if any, in the posterior crossveins of each fly in a sample were rated with reference to the position of break and the estimated amount missing based upon fifths of crossvein length. The sum of the missing fraction on both crossveins gives a rating (r_{10}) for each fly. Expressivity is given by the mean r_{10}. Penetrance is given by the percent of flies showing crossvein interruptions. The percent of interruptions at the fourth and fifth longitudinal veins or at the center of the crossvein if they occur gives us the specificity.

RESULTS

Pupal Developmental Time

Figures 1 through 19 show the results of experiments performed to determine the mean emergence time of 6-hi, 6-lo and 5-hi pupae at the three experimental temperatures. The percent (plus and minus one standard error) of flies that emerged in each sample is plotted against time on probability graph paper. In general, the points on each graph are fairly well fitted by a straight line. Thus the distribution of emergence is approximately normal. In a few cases, more than one line can be fitted to the same points. However, they give about the same mean emergence time. This suggests that more than one group of flies may be present in a sample having the same mean but with different variances in emergence distribution.

The mean emergence time in each cvl line at each experimental temperature estimates the duration of pupal development (Table 1). There is no large difference in the developmental time between the cvl lines with the exception of 5-hi Cy^+ females at 23° C, which seemed to have a longer developmental time than the rest. That of the 5-hi Cy flies were consistently shorter than those of the 5-hi Cy^+ flies. The data of Cy^+ and Cy flies came from the same samples and both kinds were raised in the same vials under identical conditions.

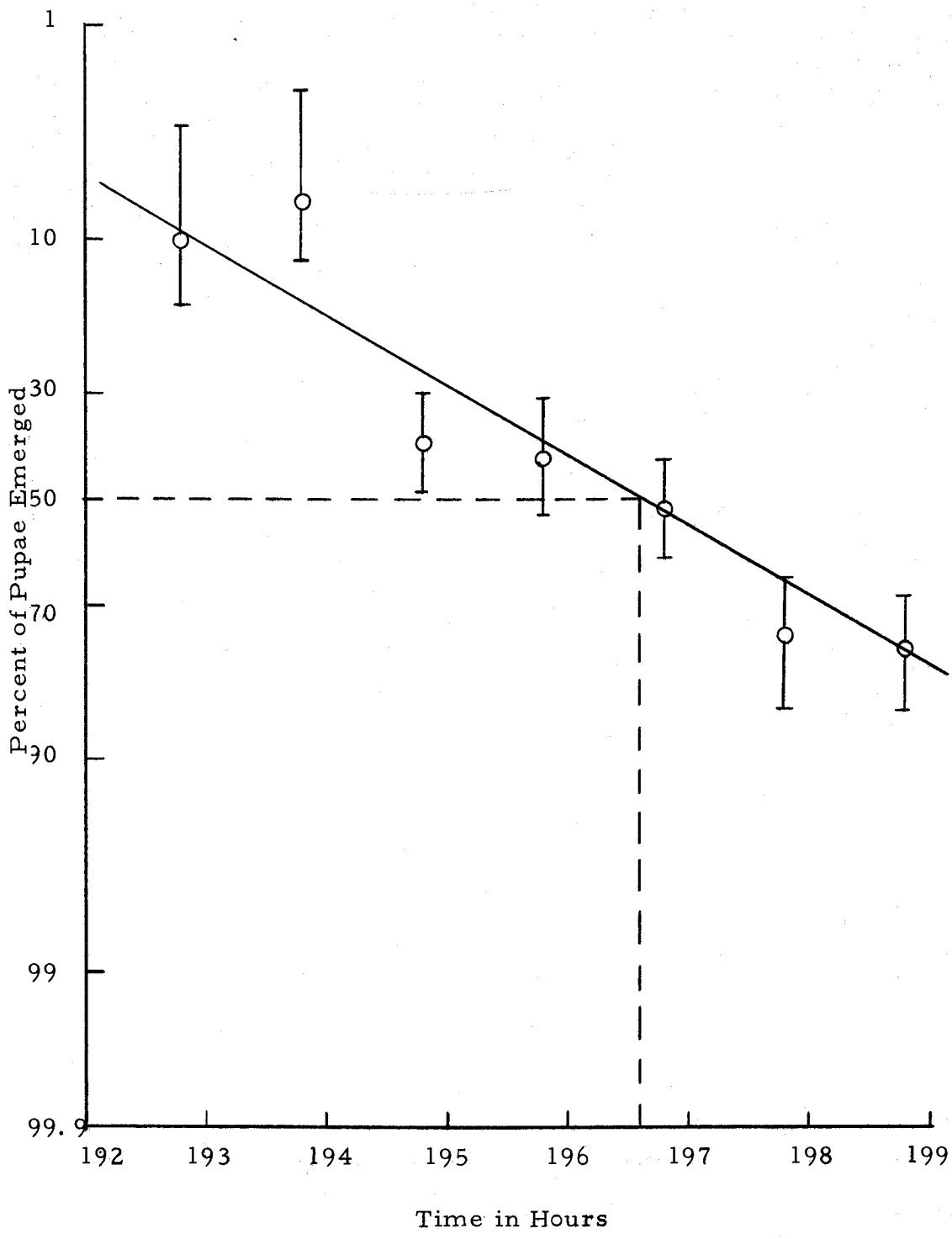

Figure 1. Developmental time of 6-hi females at 18° C.

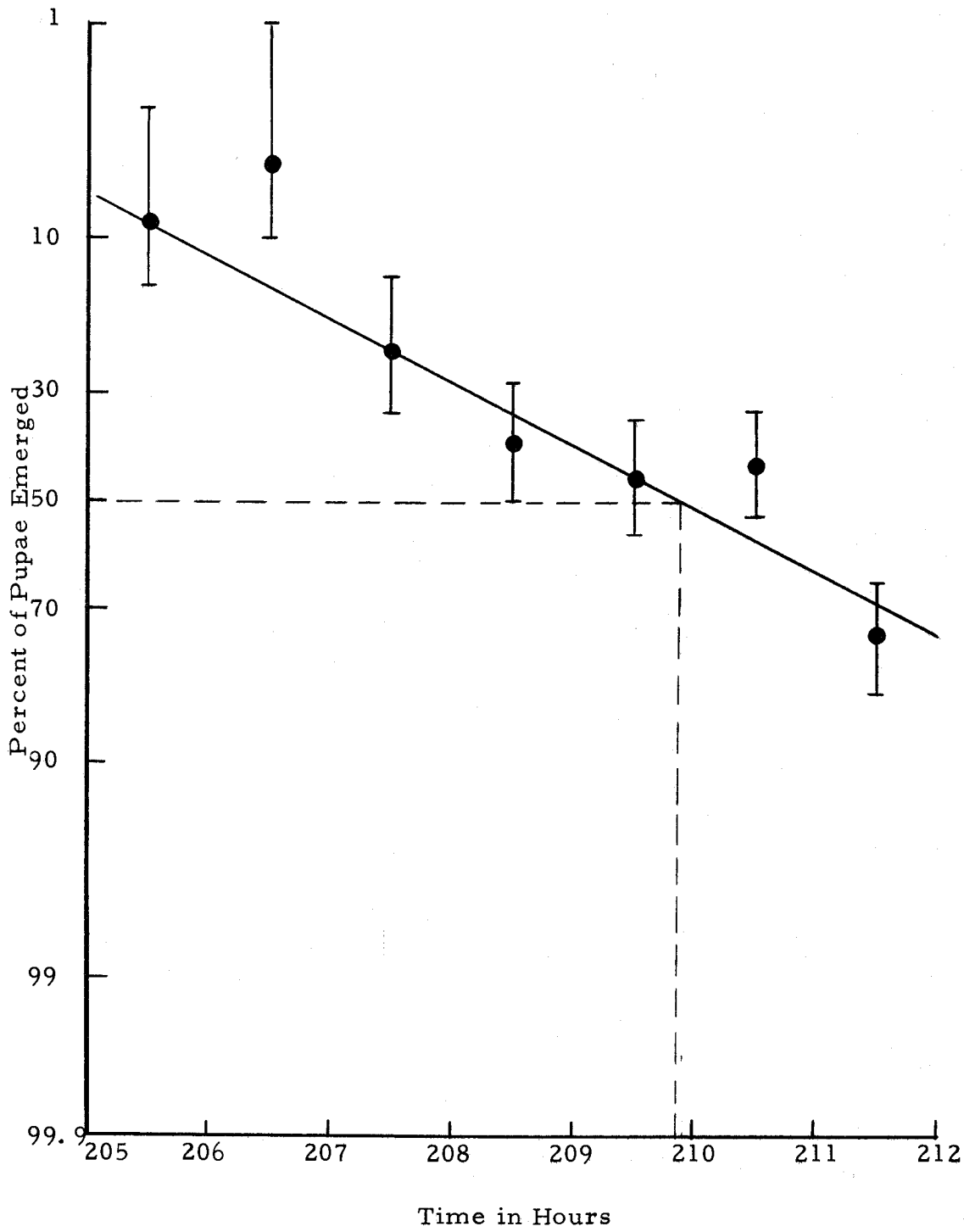

Figure 2. Developmental time of 6-hi males at 18° C.

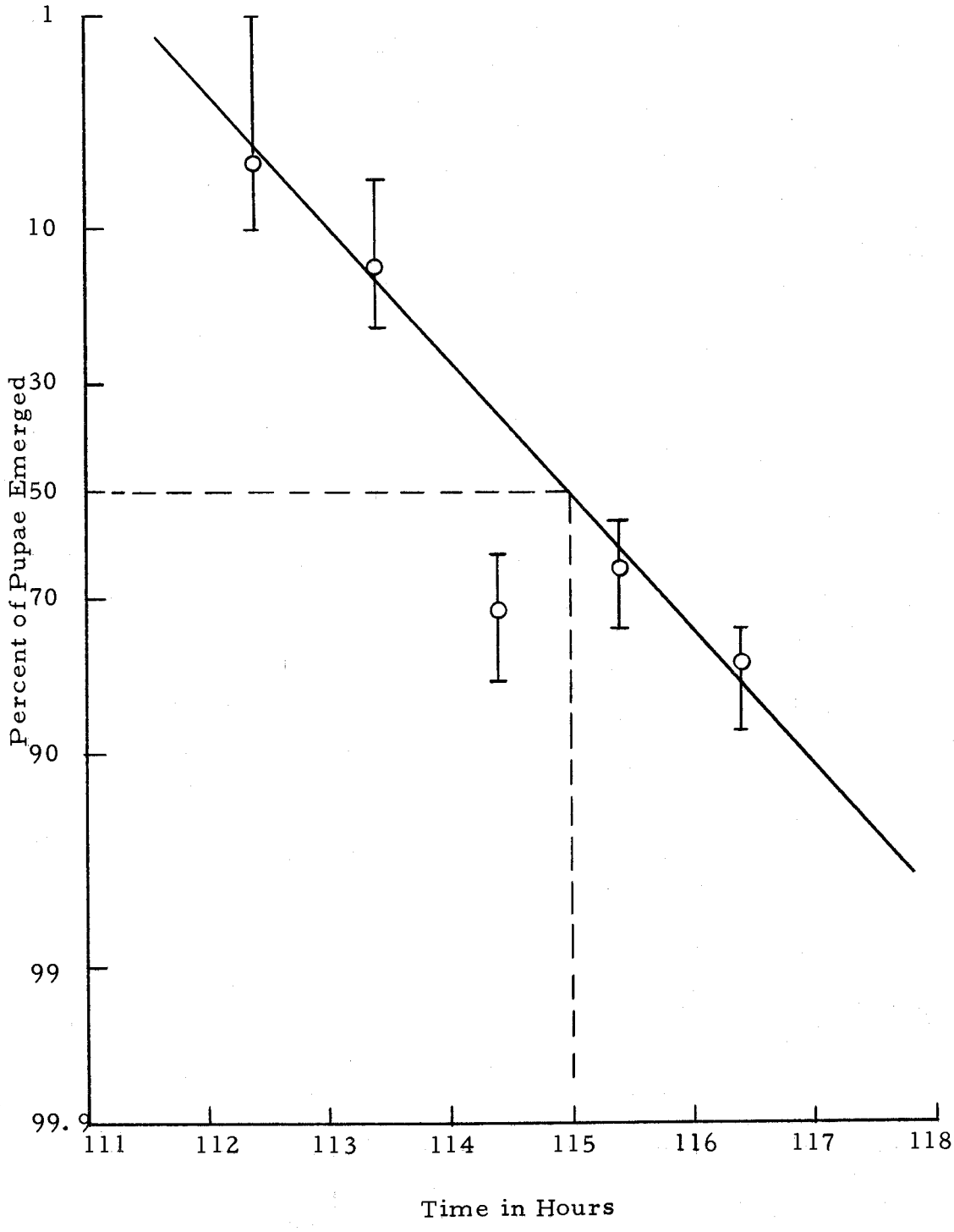

Figure 3. Developmental time of 6-hi females at 23° C.

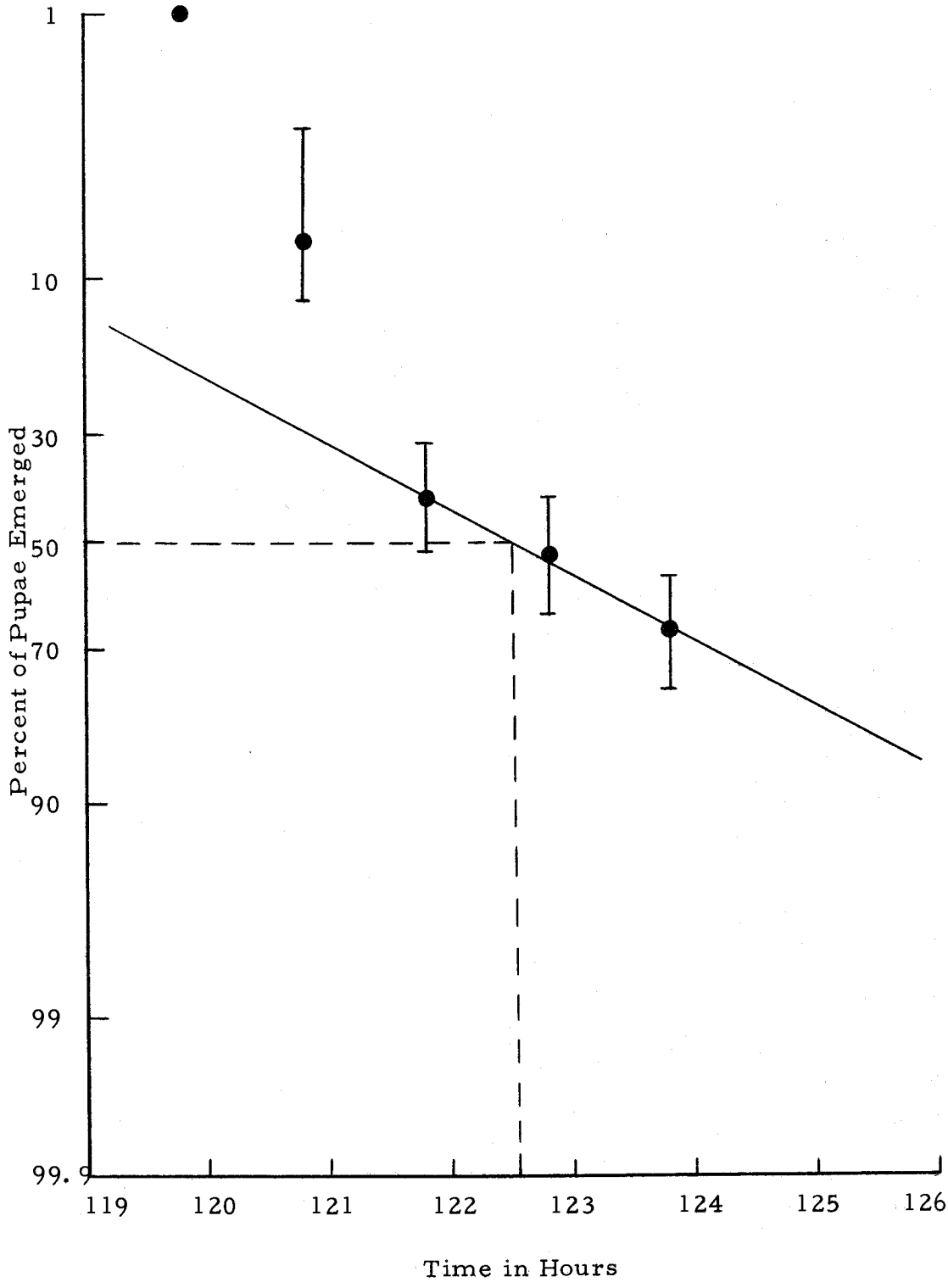

Figure 4. Developmental time of 6-hi males at 23° C.

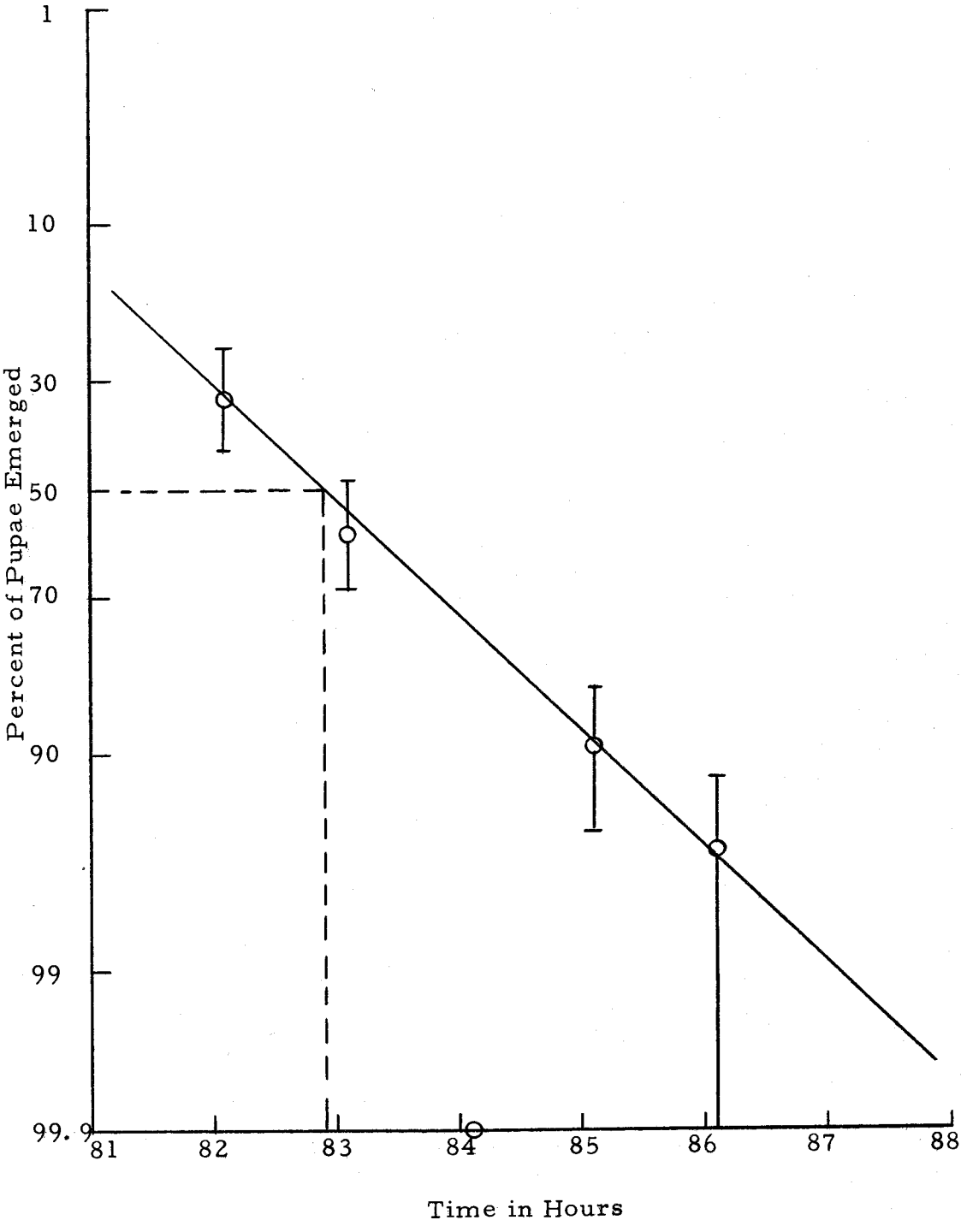

Figure 5. Developmental time of 6-hi females at 28° C.

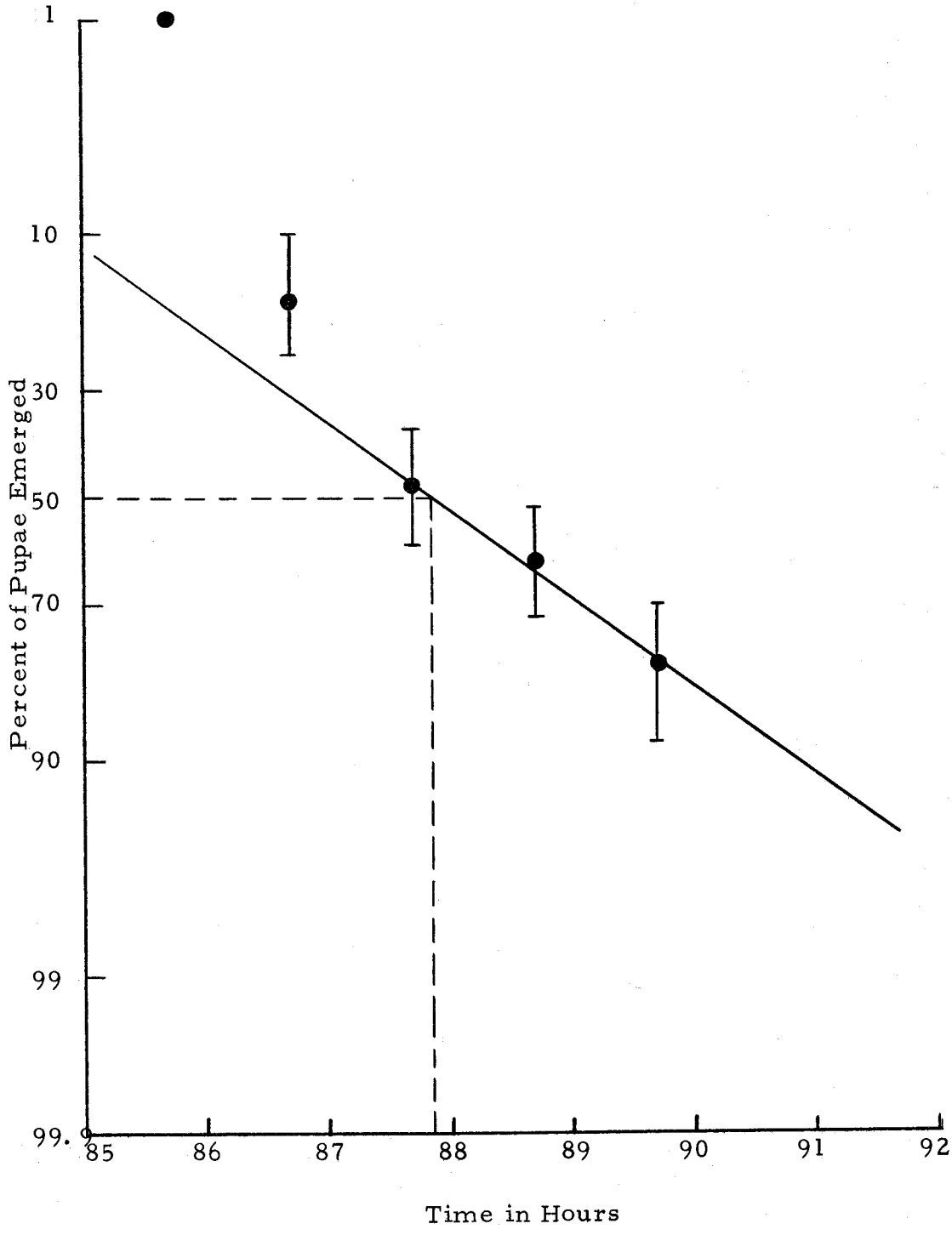

Figure 6. Developmental time of 6-hi males at 28° C.

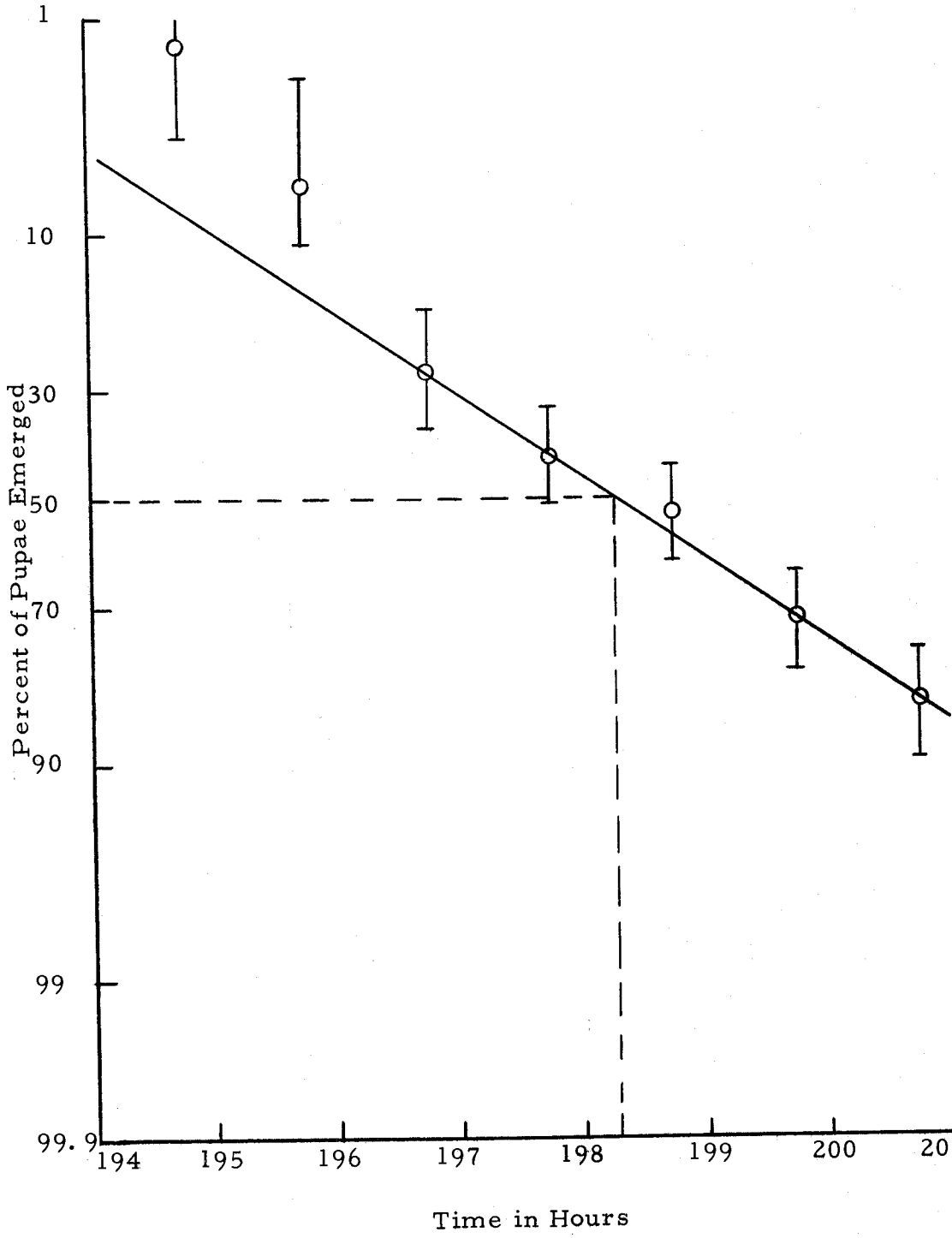

Figure 7. Developmental time of 6-lo females at 18° C.

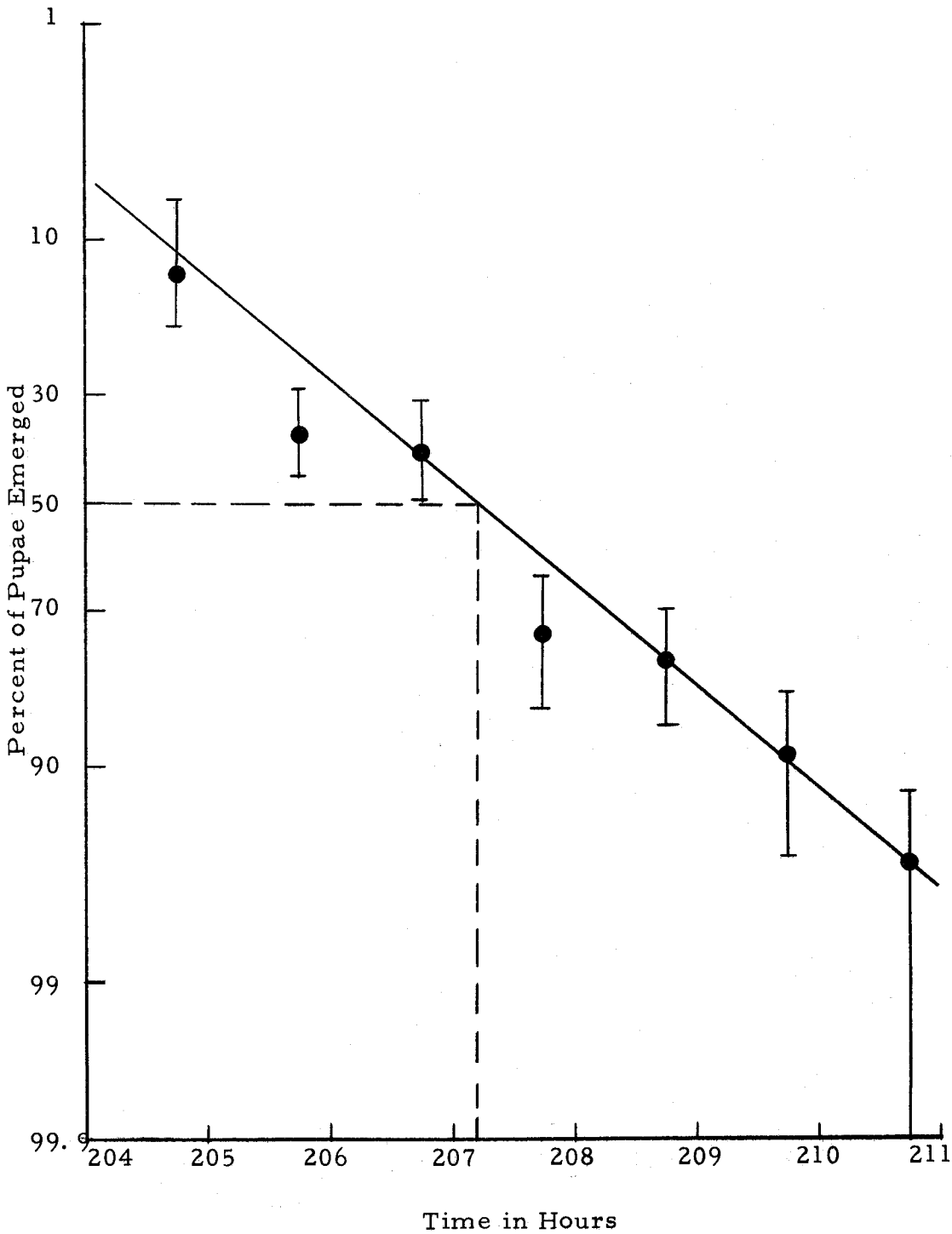

Figure 8. Developmental time of 6-lo males at 18° C.

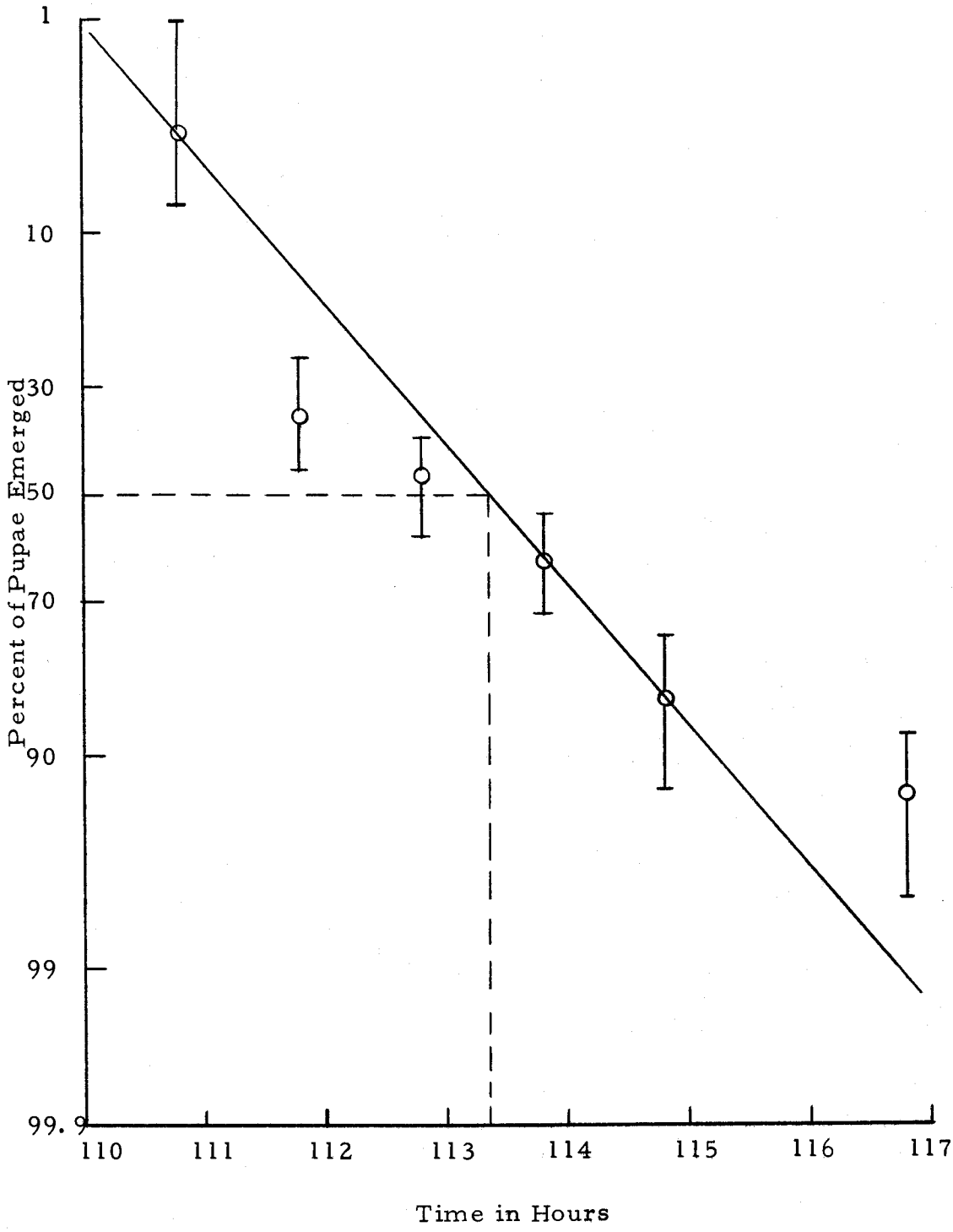

Figure 9. Developmental time of 6-lo females at 23° C.

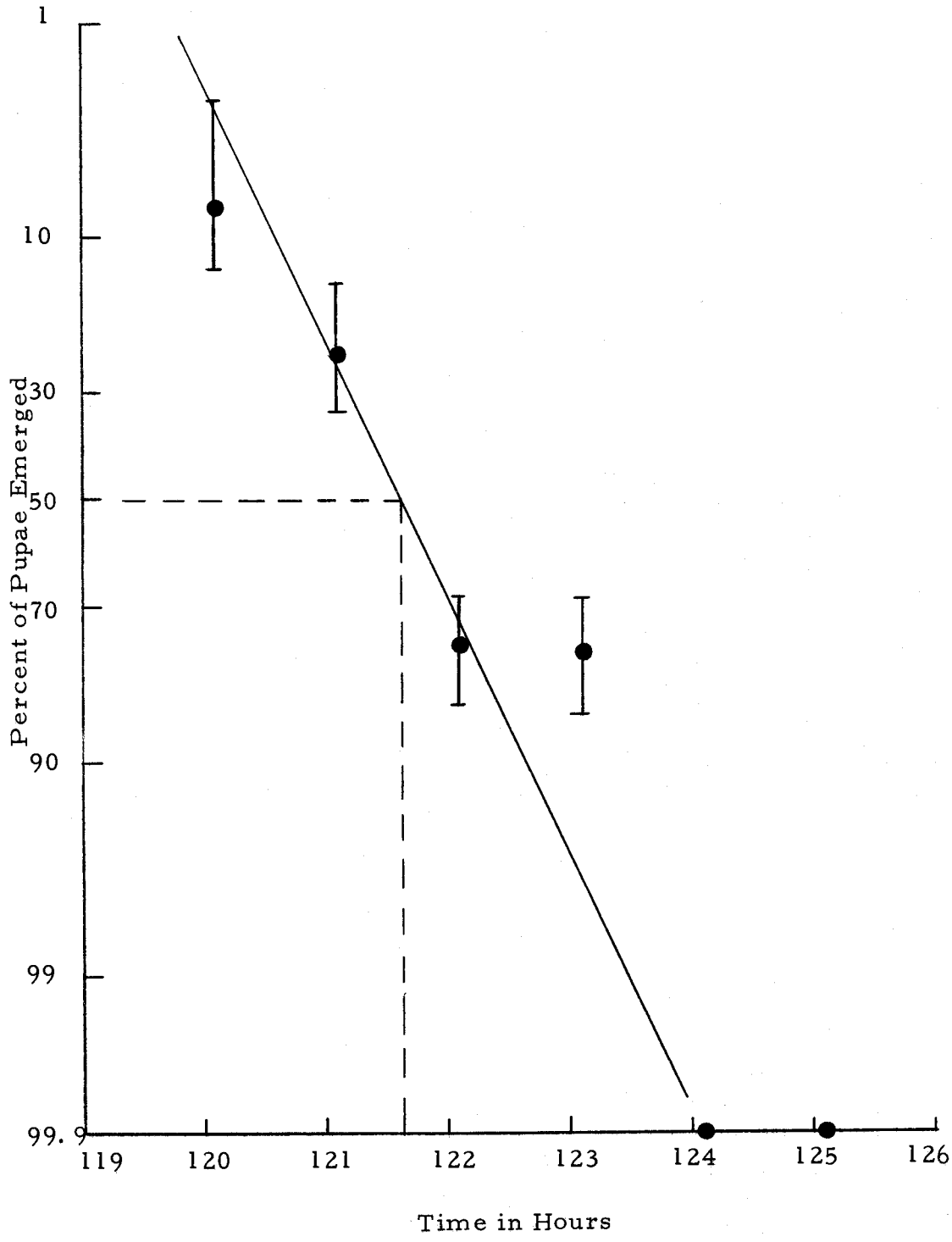

Figure 10. Developmental time of 6-lo males at 23° C.

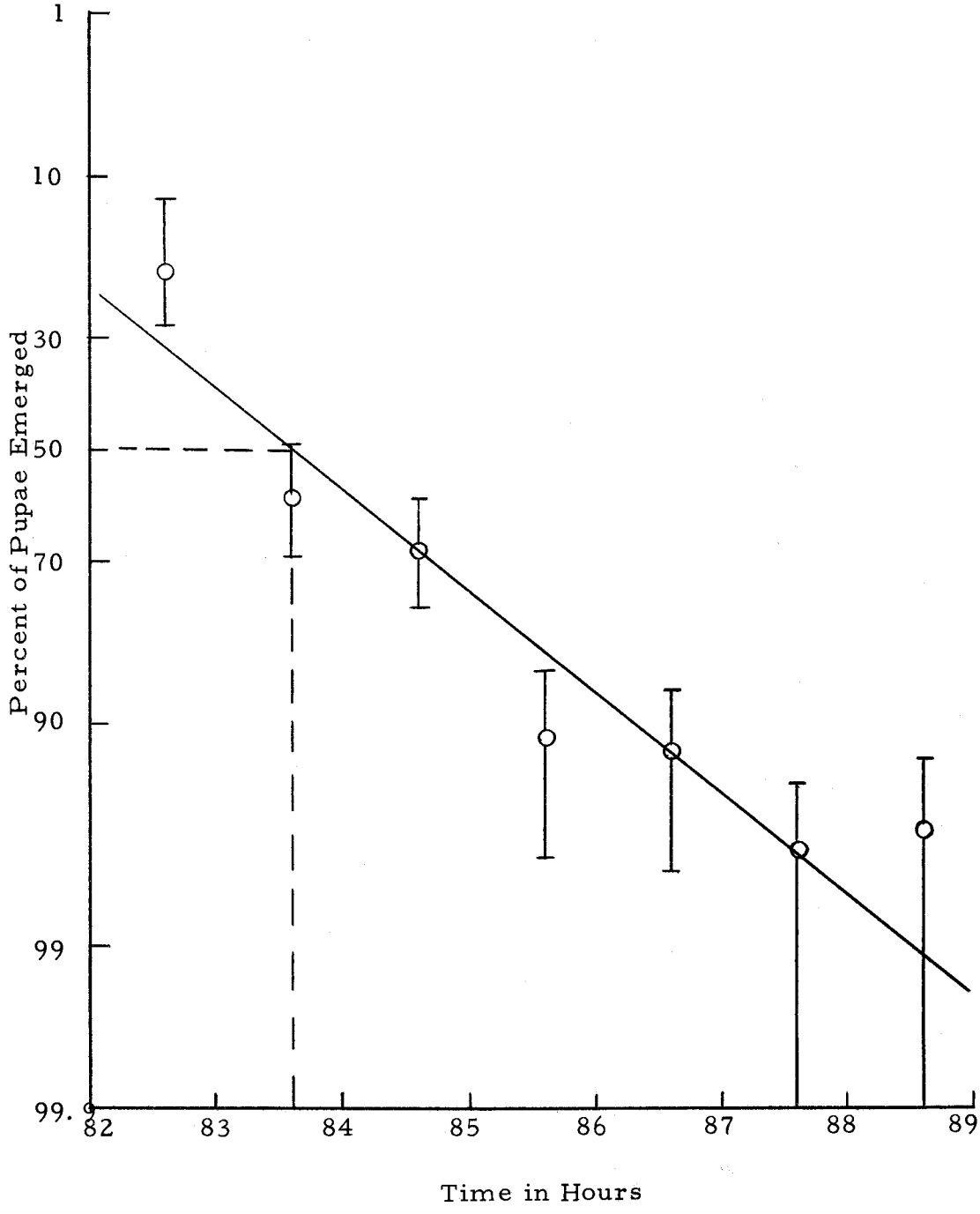

Figure 11. Developmental time of 6-lo females at 28° C.

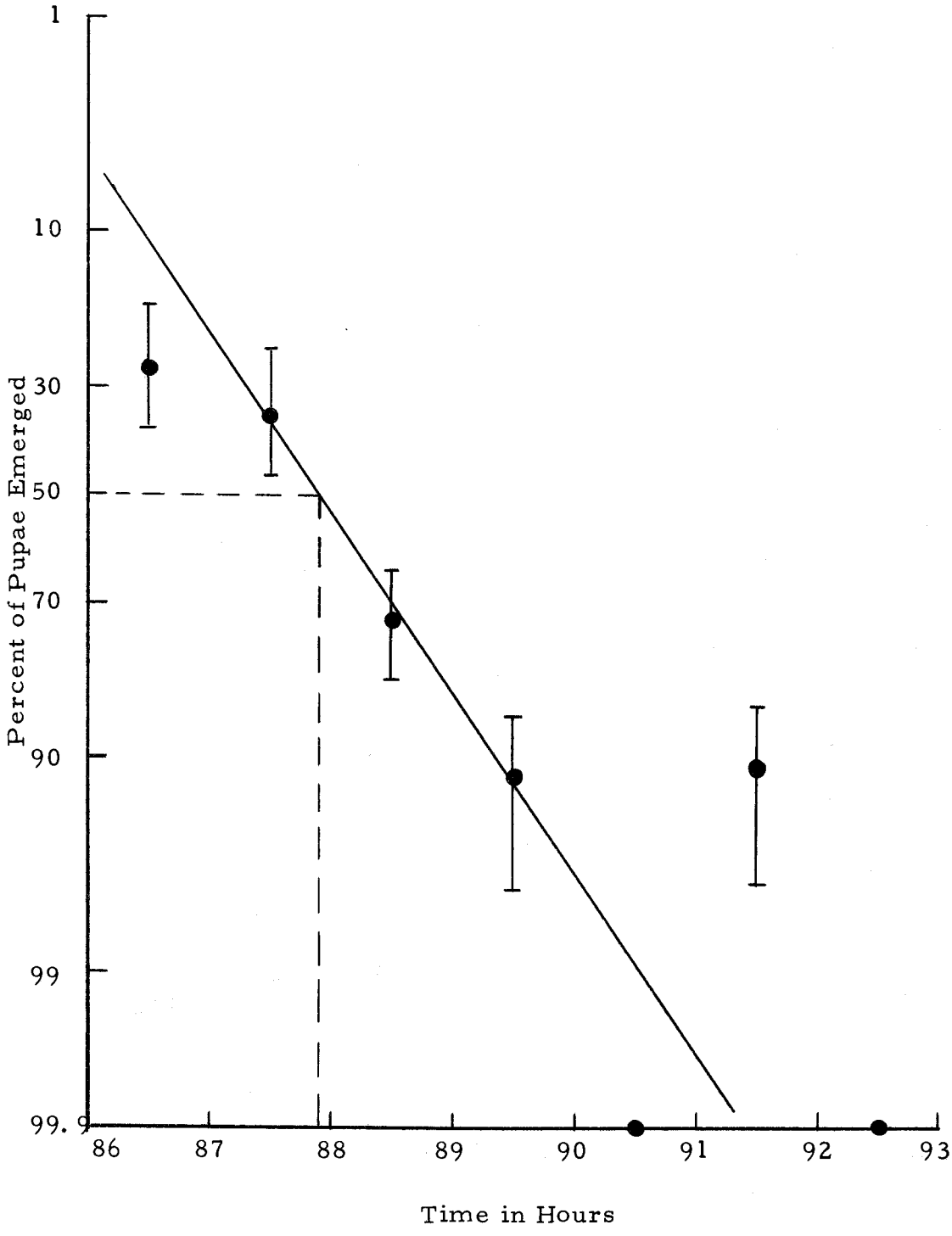

Figure 12. Developmental time of 6-lo males at 28° C.

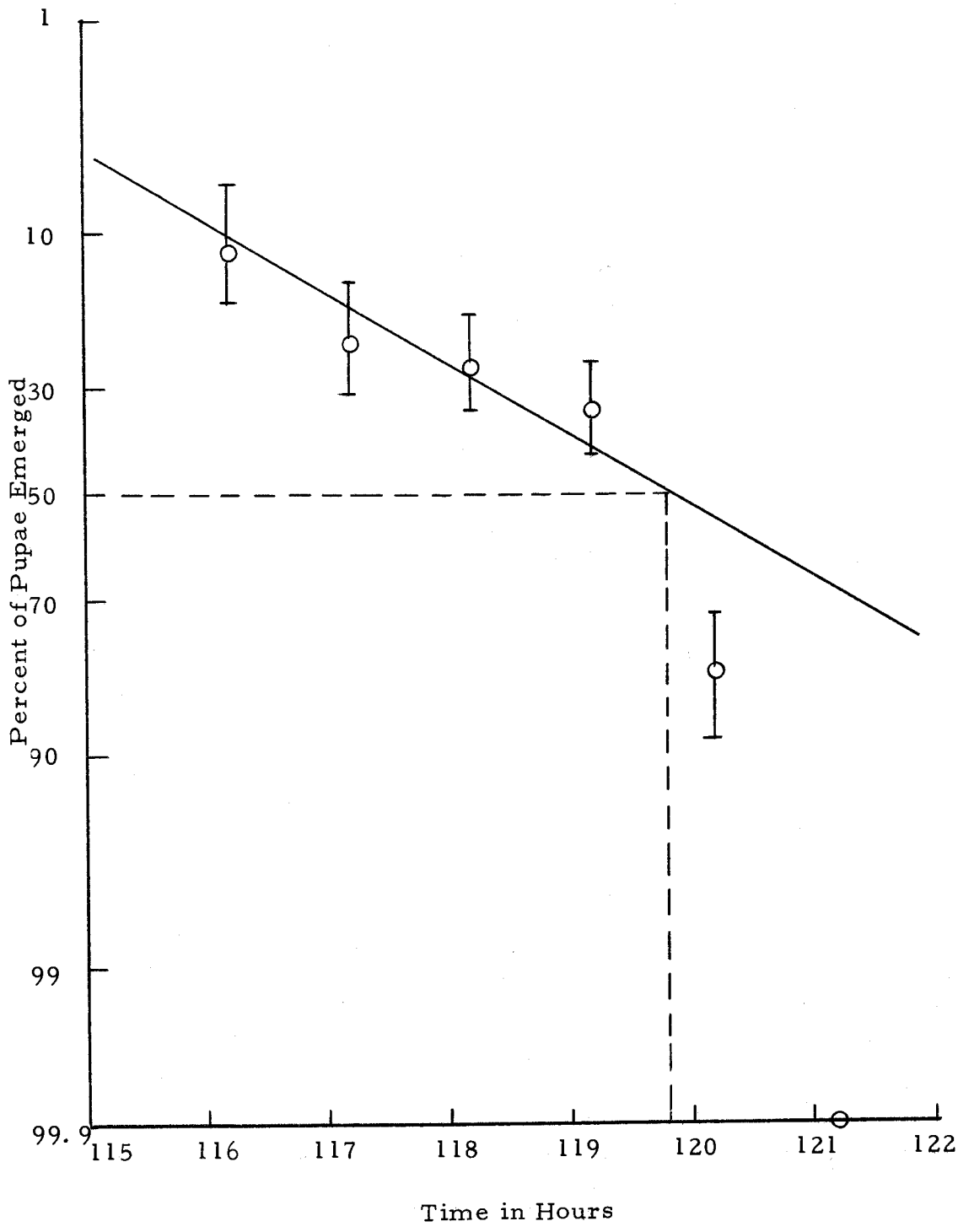

Figure 13. Developmental time of 5-hi Cy^{+} females at 23° C.

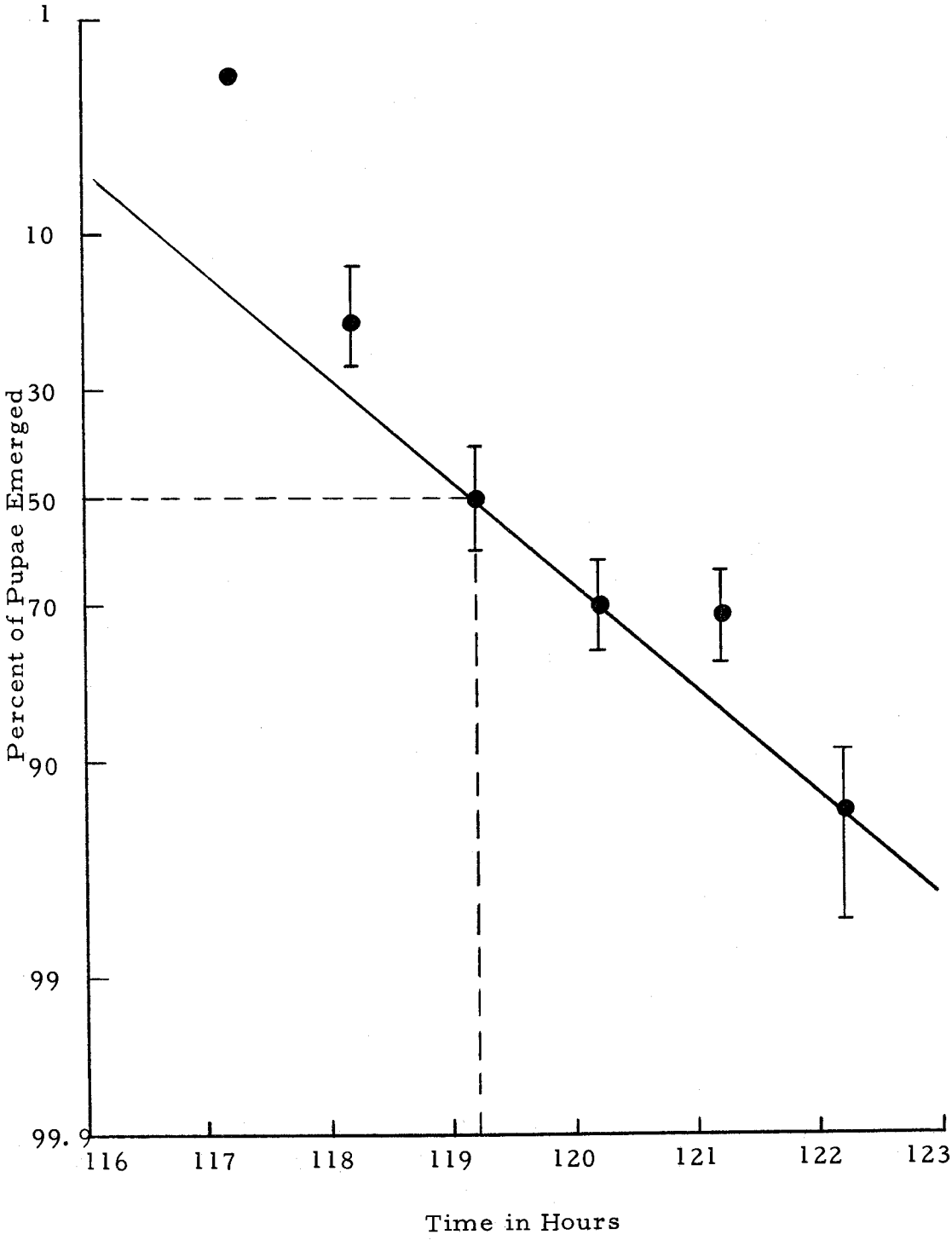

Figure 14. Developmental time of 5-hi Cy^{+} males at 23° C.

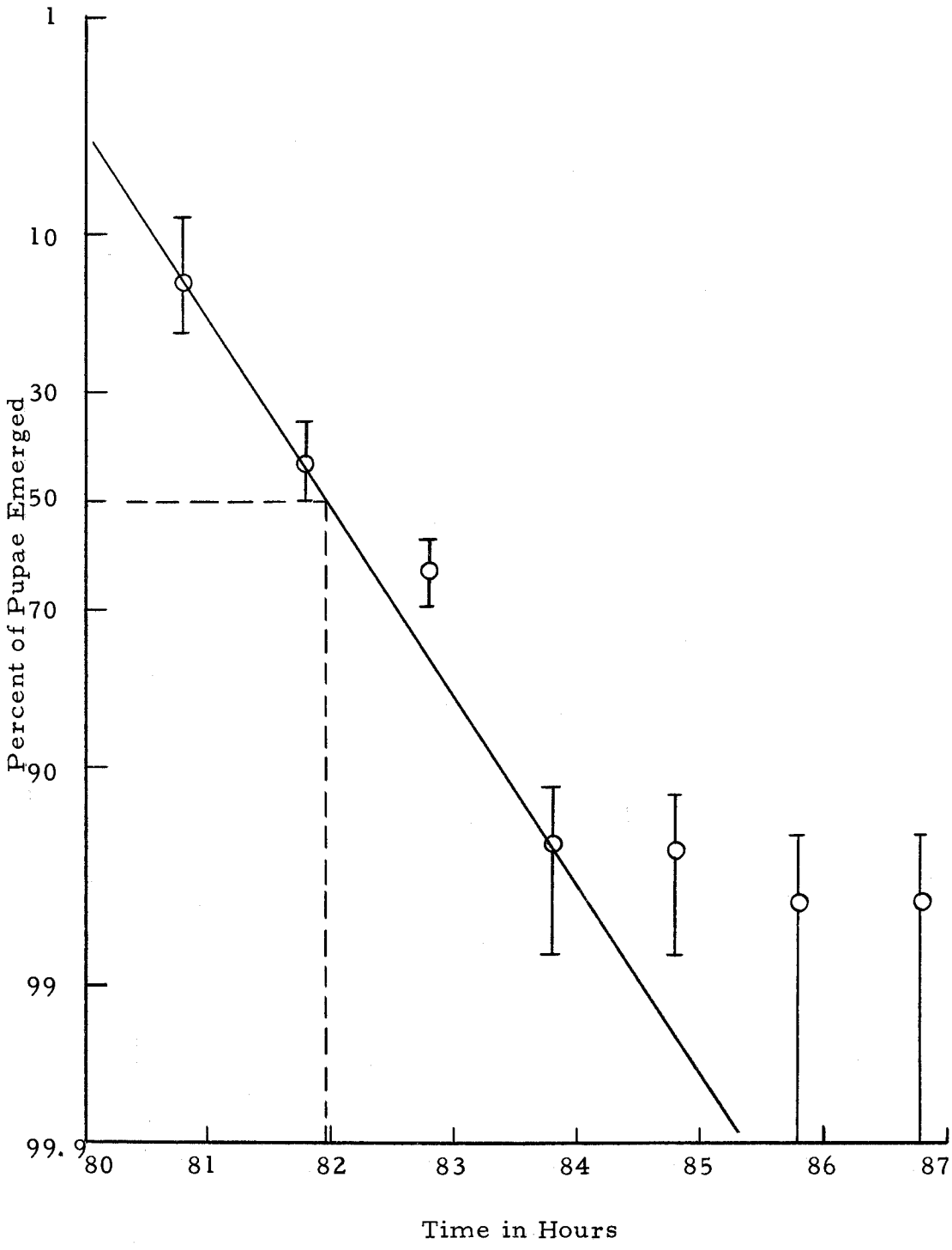

Figure 15. Developmental time of 5-hi Cy^{+} females at 28° C.

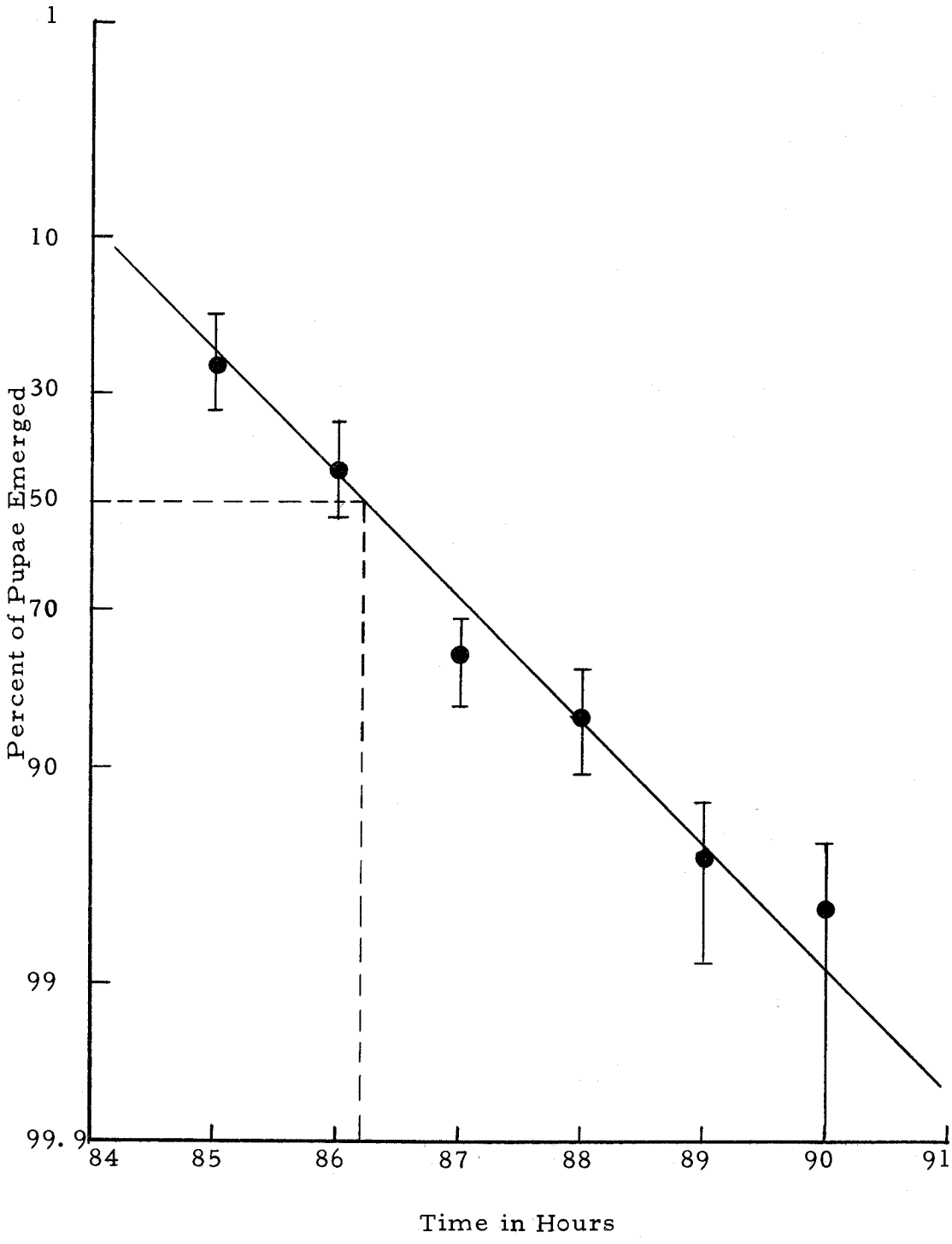

Figure 16. Developmental time of 5-hi Cy^{+} males at 28° C.

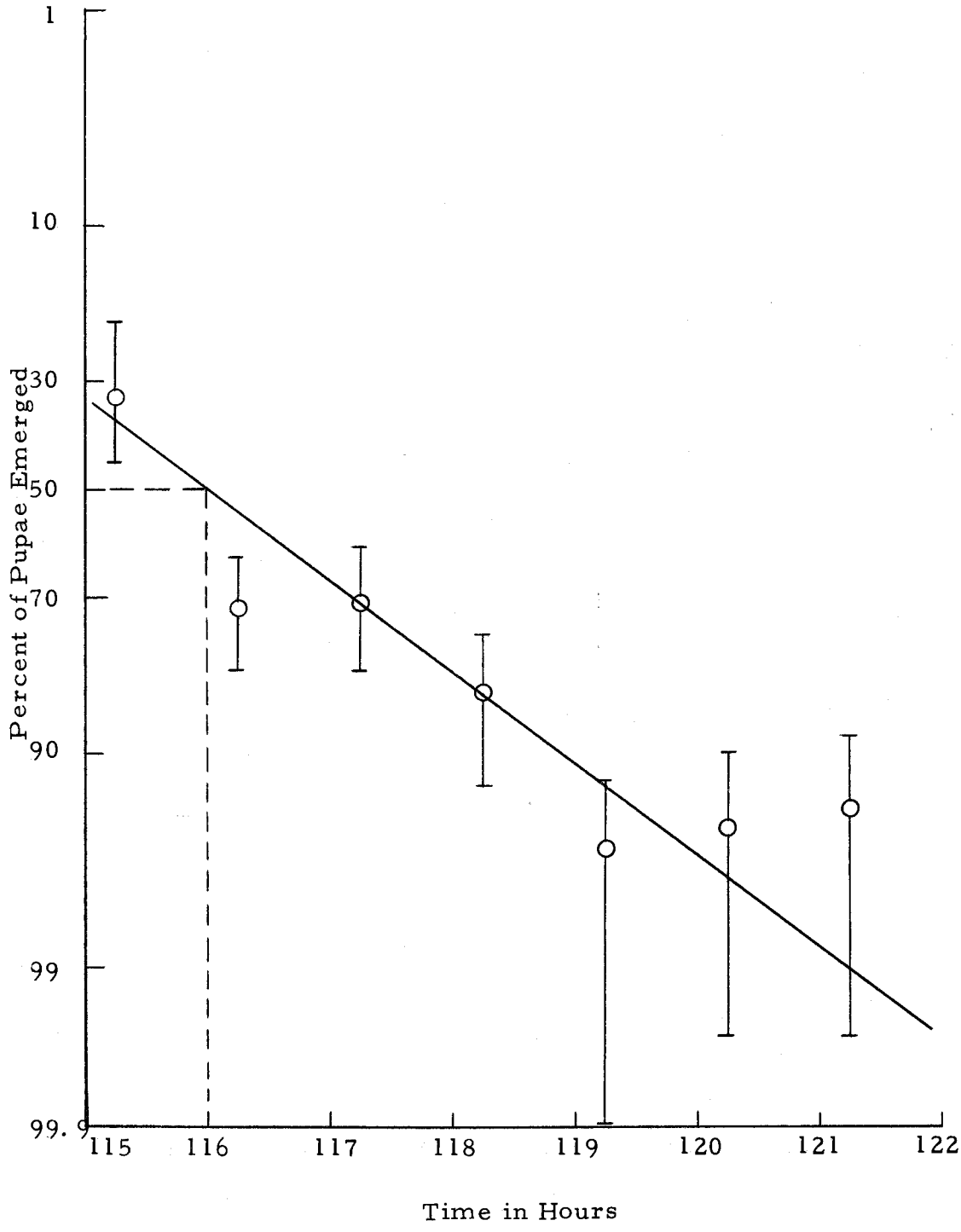

Figure 17. Developmental time of 5-hi Cy females at 23° C.

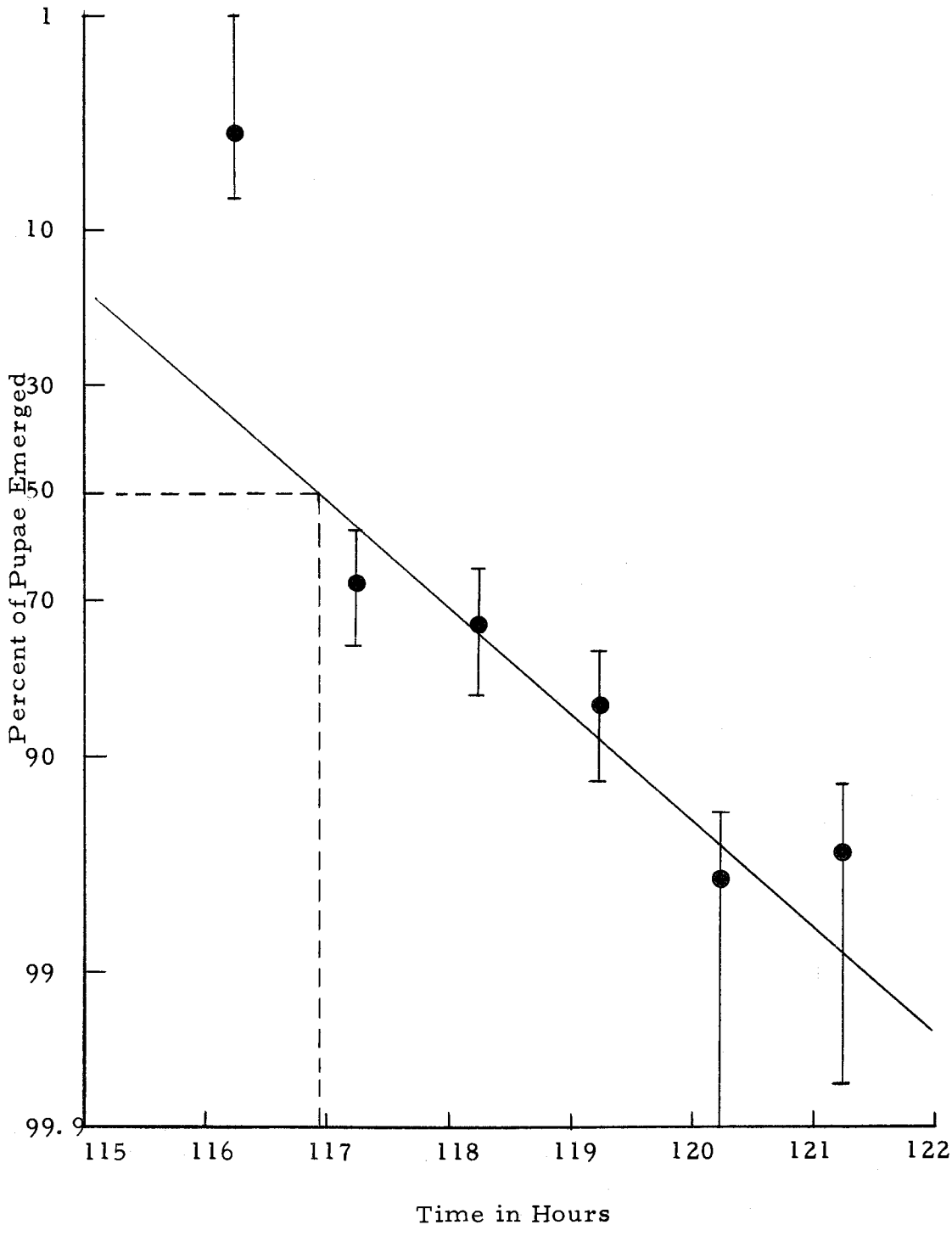

Figure 18. Developmental time of 5-hi Cy males at 23° C.

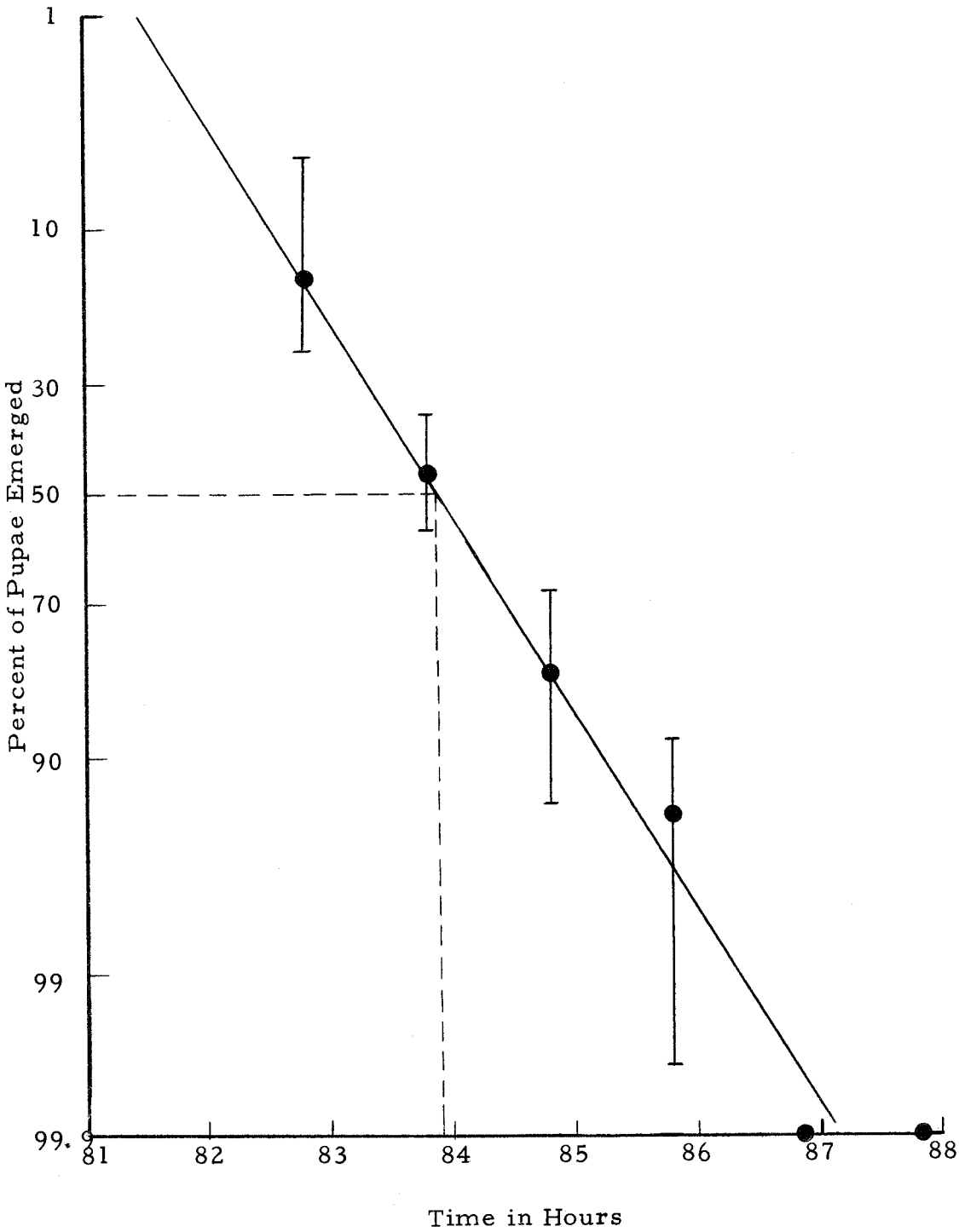

Figure 19. Developmental time of 5-hi Cy males at 28° C.

Since the Cy flies differ from the Cy^+ flies only in a second chromosome, the difference in developmental time between the two suggests that Curly chromosome carries genes that speed development.

Table 1. Pupal developmental time at 18°, 23°, and 28° C.

Line	Temp.	Time in hours ♀♀	♂♂
6-hi	18	196½	210
	23	115	122½
	28	83	88
6-lo	18	198¼	207¼
	23	113½	121½
	28	83½	88
5-hi Cy^+	18	---	---
	23	120	119¼
	28	82	86¼
5-hi Cy	18	---	---
	23	116	117
	28	<80*	84

*Youngest sample has 83.5 percent emergence at 80 hours.

With the exception of 5-hi Cy^+ and 5-hi Cy at 23° C, all flies showed a sex difference in developmental time. Males always emerged later than the females at all three experimental temperatures.

It should be pointed out here that variation in the mean emergence time is one of the sources of error when defining T. E. P. as

a fraction of pupal life. It has been demonstrated in Drosophila pupae that the rate of pupal development is critically influenced by the environmental light cycle (Pittendrigh 1954, Harker 1965). Because the light condition was not controlled during the experiments described here, it may therefore be an important source of variation, in addition to that due to sampling, in the mean emergence time.

Pupal Wing Vein Development and Determination of a Q_5 in 6-Hi

Table 2 shows the comparison of pupal wing vein morphology to the standard developmental grade (see Figure 20). From these data, the mean times to the standard grade at 23° C and at 28° C are estimated to be 29 hours and $21\frac{1}{2}$ hours respectively. Thus the temperature coefficient is 1. 35. Those calculated from the durations of pupal development at 23° and 28° C are 1. 38 for the males and 1. 39 for the females. Hence we see that the rate for early morphological development is like that for the overall development. Assuming that the early morphological and physiological rates of development are the same, we would be justified to refer the physiological development of the crossvein to overall development in order to compare rates at different temperatures. We are also assuming that this is true in 6-lo and 5-hi flies as well.

Figure 20. Pupal wing vein of cvl-6b: 24 hours at 25° C.

Table 2. Developmental grades of 6-hi pupal wing vein at 23° and 28° C.

Temp.	Age (hours)	Developmental grade*		
		-1	0	+1
23° C	28	10	0	0
	29	0	6	4
	30	0	0	10
28° C	20	10	0	0
	21	9	0	1
	22	0	2	8

*As defined in text 0, -1 and +1 represent developmental grades indistinguishable from, prior to and beyond the standard grade (see figure 20) respectively.

Cvl Phenotypes

Information of the phenotypic expressions of 6-hi, 6-lo and 5-hi at the three experimental temperatures is provided by the control samples (that is, the flies raised at constant temperature) from the several experiments performed to determine T. E. P. 's. These are brought together in Tables 3 and 4. The experiment numbers indicate the sequence of experiments done in the specified cvl line.

6-hi Phenotypes. Expressivity (r_{10}) varied with temperature. In the females, the r_{10} at 23° C was higher than the two similar r_{10}'s at 18° and 28° C. In the males, the r_{10} at 28° C was lower than the two similar r_{10}'s at 18° and 23° C. The expressivity of males at 18° C was higher than that of the females and the reverse was true at 23° and 28° C. Penetrance (P) was maximal (100 percent) at all

Table 3. Phenotypic expression of 6-hi and 6-lo at 18°, 23° and 28° C.

Line	Experiment No.	Temp.	♀♀ n	♀♀ $\bar{F}_{10}$	♀♀ P(%)	♀♀ L4(%)*	♂♂ n	♂♂ $\bar{F}_{10}$	♂♂ P(%)	♂♂ L4(%)*
6-hi	1	18	126	6.9	100	50	60	7.7	100	51
		23	101	8.0	100	51	44	7.6	100	50
		28	106	7.1	100	49	43	5.5	100	39
	2	18	55	7.7	100	50	45	8.5	100	50
		23	52	8.3	100	50	55	8.2	100	50
		28	53	8.0	100	52	51	5.6	100	36
	3	23	63	8.8	100	50	75	8.3	100	50
		28	71	8.0	100	50	73	6.0	100	50
	4	23	91	8.6	100	50	60	7.8	100	50
		28	50	8.5	100	50	51	5.9	100	50
6-lo	1	18	64	2.7	83	96	50	1.6	58	98
		23	49	0.9	45	55	64	0.2	11	80
		28	58	0.5	28	6	63	0	2	0
	2	18	84	1.8	63	86	64	1.2	53	97
		28	75	0.2	13	0	84	0.1	2	0
	3	18	150	2.7	86	87	101	1.6	58	90
		28	86	0.2	11	10	72	0	0	--

*Calculated from the total number of interruptions.

Table 4. Phenotypic expression of 5-hi Cy$^+$ and 5-hi Cy at 18°, 23° and 28°C.

Line	Experiment No.	Temp	♀♀ n	♀♀ $\bar{r}_{10}$	♀♀ P(%)	♀♀ L4(%)*	♂♂ n	♂♂ $\bar{r}_{10}$	♂♂ P(%)	♂♂ L4(%)*
5-hi Cy$^+$	1	18	52	3.8	90	59	80	0.2	13	33
		23	49	5.4	98	43	56	2.4	84	29
		28	15	0.9	53	91	41	0.1	7	0
	2	23	83	4.8	100	35	62	2.3	90	21
		28	81	2.0	91	15	61	0.4	34	8
	3	23	100	5.1	100	39	87	2.0	89	20
		28	56	2.6	91	23	77	0.9	57	13
5-hi Cy	1	18	33	0.7	36	93	16	0	0	0
		23	29	3.4	97	43	39	0.7	41	32
	2	23	61	2.4	90	35	70	0.6	43	11
		28	61	1.0	62	24	38	0.1	5	50
	3	23	37	2.7	90	45	37	0.8	49	8
		28	27	1.0	67	13	37	0.2	16	30

*Calculated from total number of interruptions.

temperatures. Specificity is described by the fraction of the interruptions that are at the fourth longitudinal vein (L4). Specificity was 50 percent at all temperatures in both sexes. The phenotypes were fairly consistent between different control groups.

6-lo Phenotypes. Expressivity, penetrance and specificity all varied inversely with temperature in both sexes. The females had a higher r_{10} and penetrance than the males at all temperatures. Sepcificity was about the same in both sexes. The phenotypes were quite consistent between different control groups, with the possible exception of the female sample raised at 18° C in experiment 2, which appeared to have a lower r_{10} and penetrance than those raised at the same temperature in experiments 1 and 3.

5-hi Phenotypes. Expressivity, penetrance and specificity varied with temperature in both Cy^+ and Cy flies. In the Cy^+ females, the r_{10} at 18° C was higher than that at 28° C and lower than that at 23° C. In Cy^+ males, Cy females and Cy males, the r_{10} at 23° C was higher than the two similar r_{10}'s at 18° and 28° C. In all flies, penetrance was higher at 23° than at 28° C. No conclusion can be drawn about specificity in Cy males since penetrance was very low in these flies. Expressivity and penetrance were higher in females than in males at all temperatures. Penetrance and expressivity were always higher in Cy^+ than in Cy, thus the Cy and Cy^+ chromosomes differ in modifiers as reported by Mohler (1965b). Phenotypes of different

control groups were quite constant.

Preliminary T. E. P. Determination

Tables 5, 6 and 7 show the results of the preliminary experiments in T. E. P. determination. With the exception of 6-hi flies transferred between 18° and 23° C, among which no phenotypic differences were evident, the T. E. P. in each cvl line can be recognized to be in the period between one-eighth and one-quarter of pupal life.

Table 5. Preliminary determination of T. E. P. in 6-hi.

% of pupal life spent in one temp. before transfer	Change of Temp.	♀♀		♂♂	
		n	r_{10}	n	r_{10}
100*	18 -no change	55	7. 7	45	8. 5
50	18 to 23	47	7. 7	48	8. 2
25	18 to 23	42	8. 8	41	7. 5
12. 5	18 to 23	39	7. 9	48	7. 4
100*	23 -no change	52	8. 3	55	8. 2
50	23 to 18	44	8. 2	45	7. 4
25	23 to 18	51	8. 4	41	8. 8
12. 5	23 to 18	46	8. 8	47	8. 3
100*	23 -no change	101	8. 0	44	7. 6
50	23 to 28	43	8. 8	50	8. 2
25	23 to 28	41	9. 4	51	7. 6
12. 5	23 to 28	50	7. 2	5	6. 0
100*	28 -no change	106	7. 1	43	5. 5
50	28 to 23	59	7. 2	50	5. 3
25	28 to 23	50	7. 6	50	6. 5
12. 5	28 to 23	54	9. 4	60	8. 7

*Controls.

Table 6. Preliminary determination of T. E. P. in 6-lo.

% of pupal life spent in one temp. before transfer	Change of Temp.	♀♀ n	♀♀ r_{10}	♂♂ n	♂♂ r_{10}
100*	18 -no change	64	2. 7	50	1. 6
50	18 to 23	55	3. 0	39	1. 8
25	18 to 23	50	2. 7	51	1. 6
12. 5	18 to 23	44	1. 4	49	0. 3
100*	23 -no change	49	0. 9	64	0. 2
50	23 to 18	51	1. 1	47	0. 4
25	23 to 18	54	1. 1	51	0. 2
12. 5	23 to 18	53	2. 7	51	1. 2
100*	23 -no change	49	0. 9	64	0. 2
50	23 to 28	45	1. 0	56	0. 2
25	23 to 28	41	1. 2	49	0. 6
12. 5	23 to 28	52	0. 1	46	0
100*	28 -no change	58	0. 5	63	0
50	28 to 23	54	0. 1	43	0
25	28 to 23	58	0. 2	48	0
12. 5	28 to 23	43	1. 2	50	0. 2

Table 7. Preliminary determination of T.E.P. in 5-hi Cy$^+$ and 5-hi Cy.

% of pupal life spent in one temp. before transfer		Change of Temp.	♀♀ n	♀♀ r_{10}	♂♂ n	♂♂ r_{10}
5-hi Cy$^+$	100*	23 - no change	49	5. 4	56	2. 4
	50	23 to 28	55	5. 3	72	1. 0
	25	23 to 28	58	6 4	64	0. 9
	12. 5	23 to 28	46	1. 5	48	0. 2
	100*	28 -no change	15	0. 9	41	0. 1
	50	28 to 23	51	1. 8	55	0. 4
	25	28 to 23	63	1. 3	66	0. 4
	12. 5	28 to 23	53	2. 5	50	0. 6
5-hi Cy	100*	23 -no change	29	3. 4	39	0. 7
	50	23 to 28	19	4. 0	19	0. 7
	25	23 to 28	41	2. 8	34	0. 5
	12. 5	23 to 28	34	0. 5	50	0. 2
	100*	28 -no change	--	--	2	0
	50	28 to 23	39	1. 1	33	0
	25	28 to 23	34	0. 9	30	0. 1
	12. 5	28 to 23	54	1. 7	46	0. 2

*Controls.

Flies spending one-quarter or more of their pupal life at one temperature (T_1) before being transferred to the other temperature (T_2) had a r_{10} similar to flies raised continuously in T_1. Flies spending one-eighth of their pupal life in T_1 before being transferred to T_2 had a r_{10} similar to flies raised continuously in T_2. For example, 6-lo females spending one-quarter and one-half of their pupal life in 28° C before being transferred to 23° C had r_{10}'s of 0. 2 and 0. 1 respectively while 6-lo females spending one-eighth of their pupal life in 28° C before being transferred to 23° C had a r_{10} of 1. 2. Conversely, 6-lo females spending one-quarter and one-half of their pupal life at 23° C before being transferred to 28° C had r_{10}'s of 1. 2 and 1. 0 respectively while 6-lo females spending one-eighth of their pupal life at 23° C before being transferred to 28° C had a r_{10} of 0. 1 (see Table 6).

Temperature-Effective Periods

The results of hourly transfers from T_1 to T_2 and from T_2 to T_1 within the preliminary T. E. P. are plotted in Figures 21 through 28. Each point on a graph represents the mean r_{10} of a sample of flies plotted against the time of transfer. In the graphs the time is measured as fraction of pupal life spent at the first temperature. The lines were fitted by eye and are intended to illustrate the apparent changes in phenotypes.

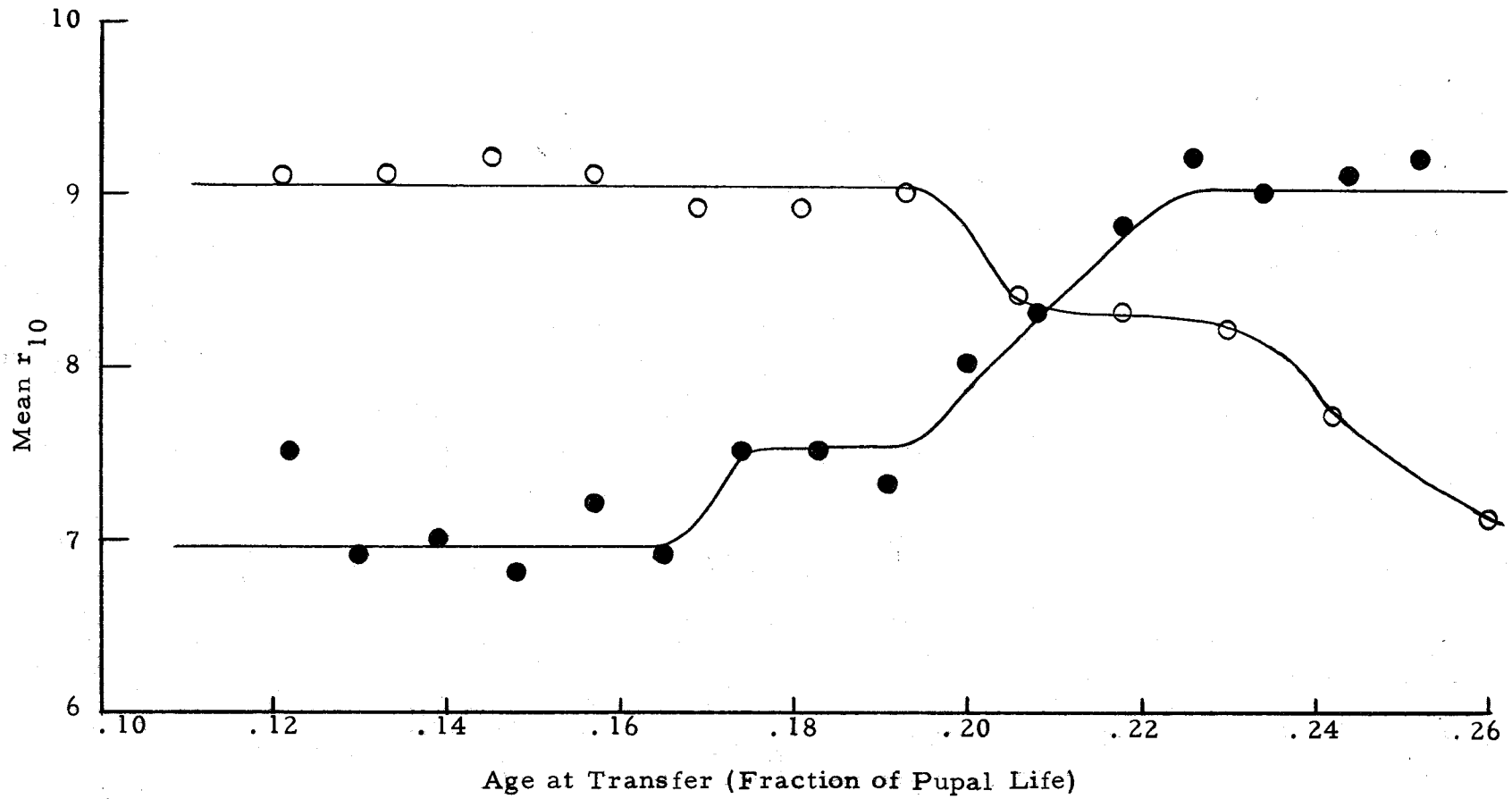

Figure 21. T. E. P. in 6-hi females at 23° and 28° C. (Open circles - transfer from 28° to 23° C, closed circles - transfer from 23° to 28° C.)

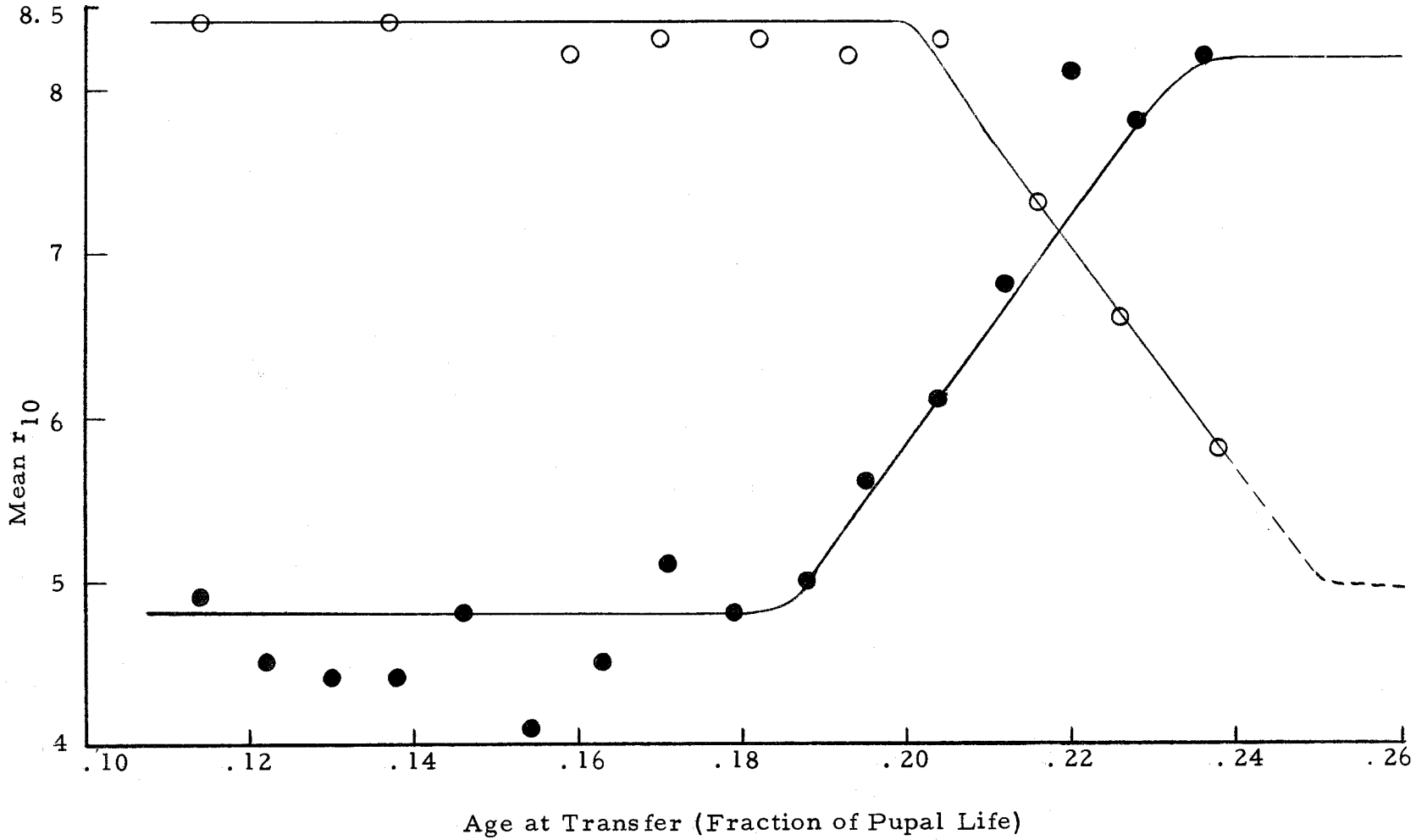

Figure 22. T. E. P. in 6-hi males at 23° and 28° C. (Open circles - transfers from 28° to 23° C, closed circles - transfers from 23° to 28° C.)

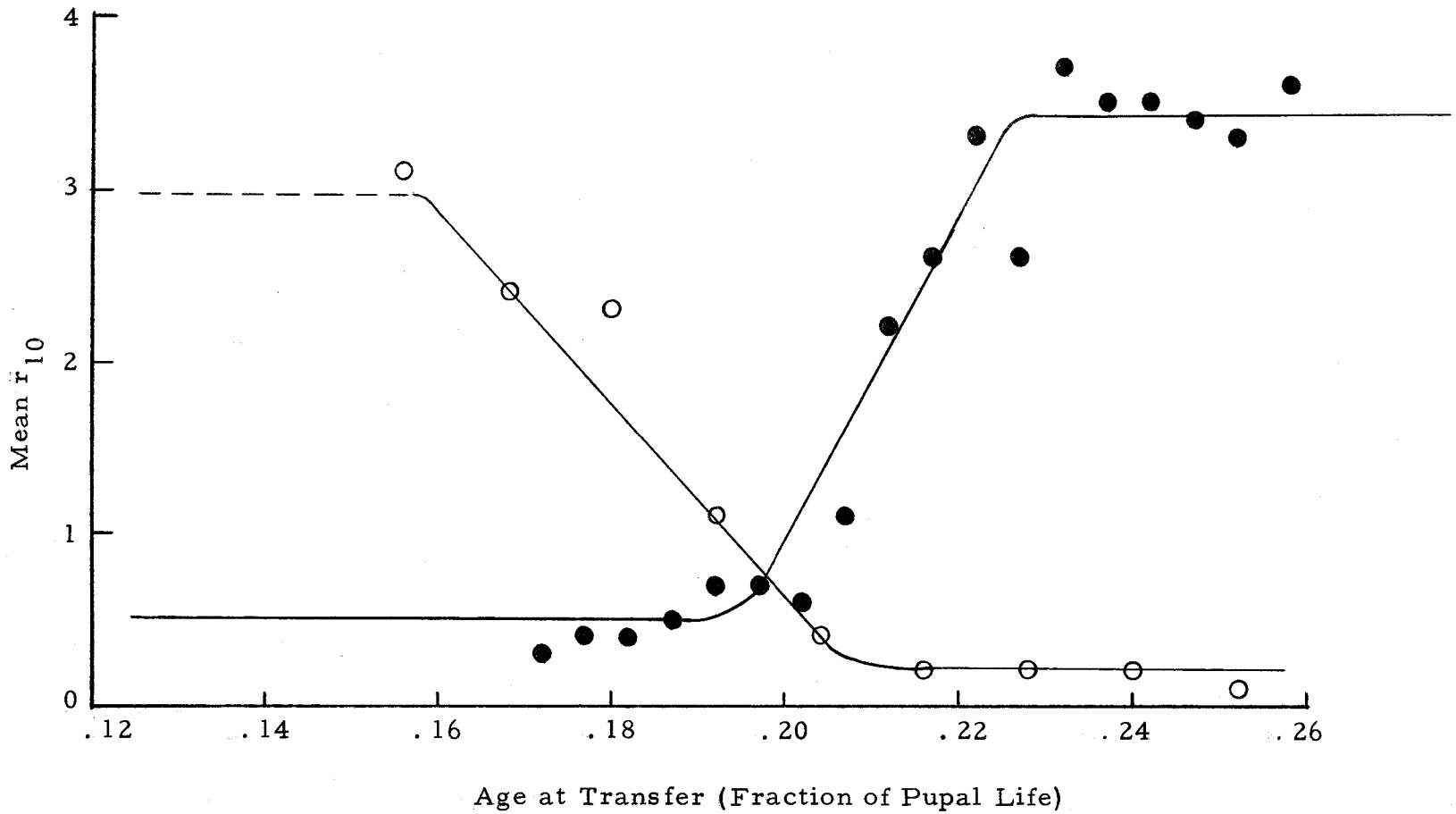

Figure 23. T. E. P. in 6-lo females at 18° and 28° C. (Open circles - transfers from 28° to 18° C, closed circles - transfers from 18° to 28° C.)

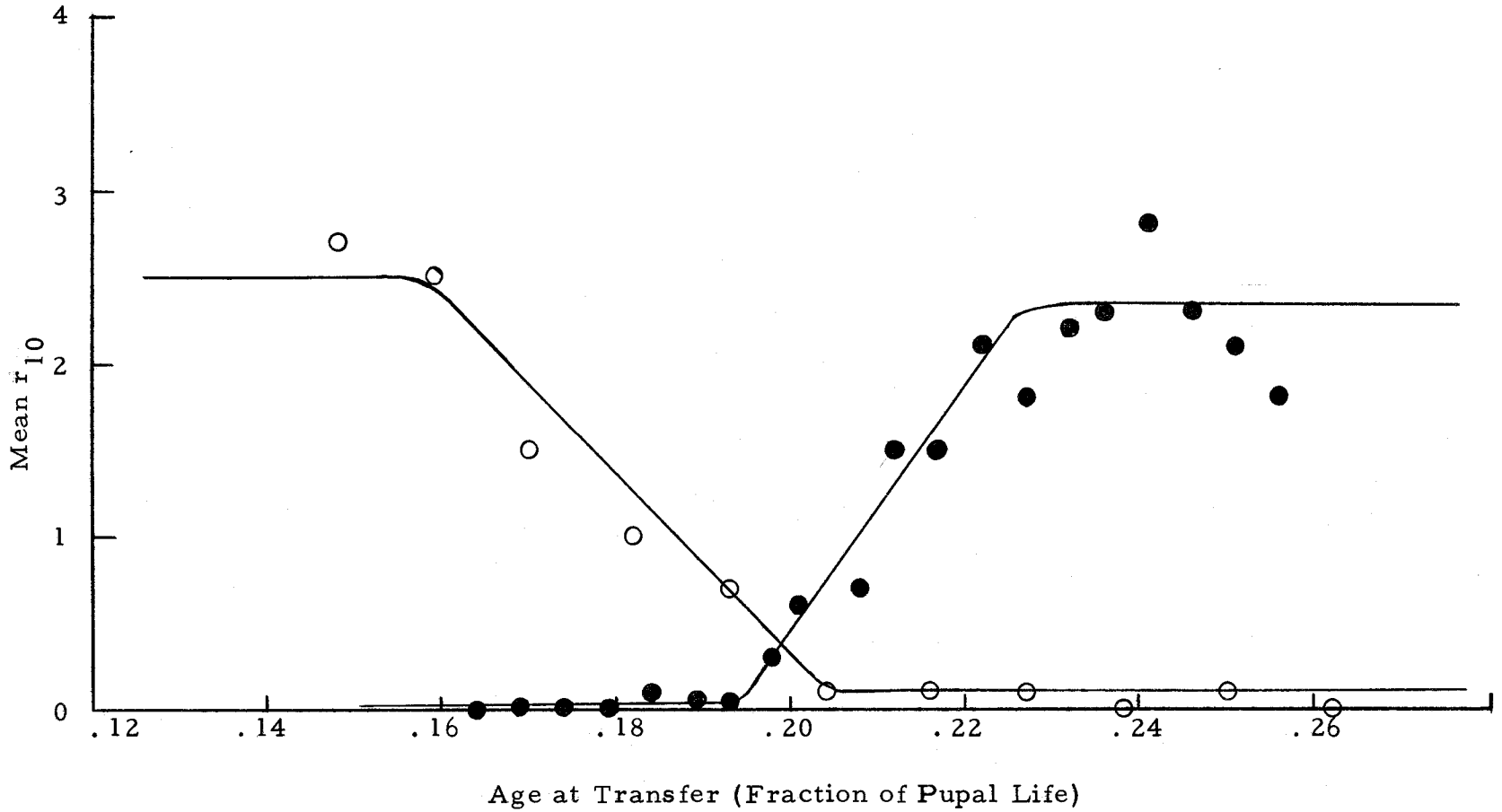

Figure 24. T. E. P. in 6-lo males at 18° and 28°C. (Open circles - transfers from 28° to 18°C, closed circles - transfers from 18° to 28°C.)

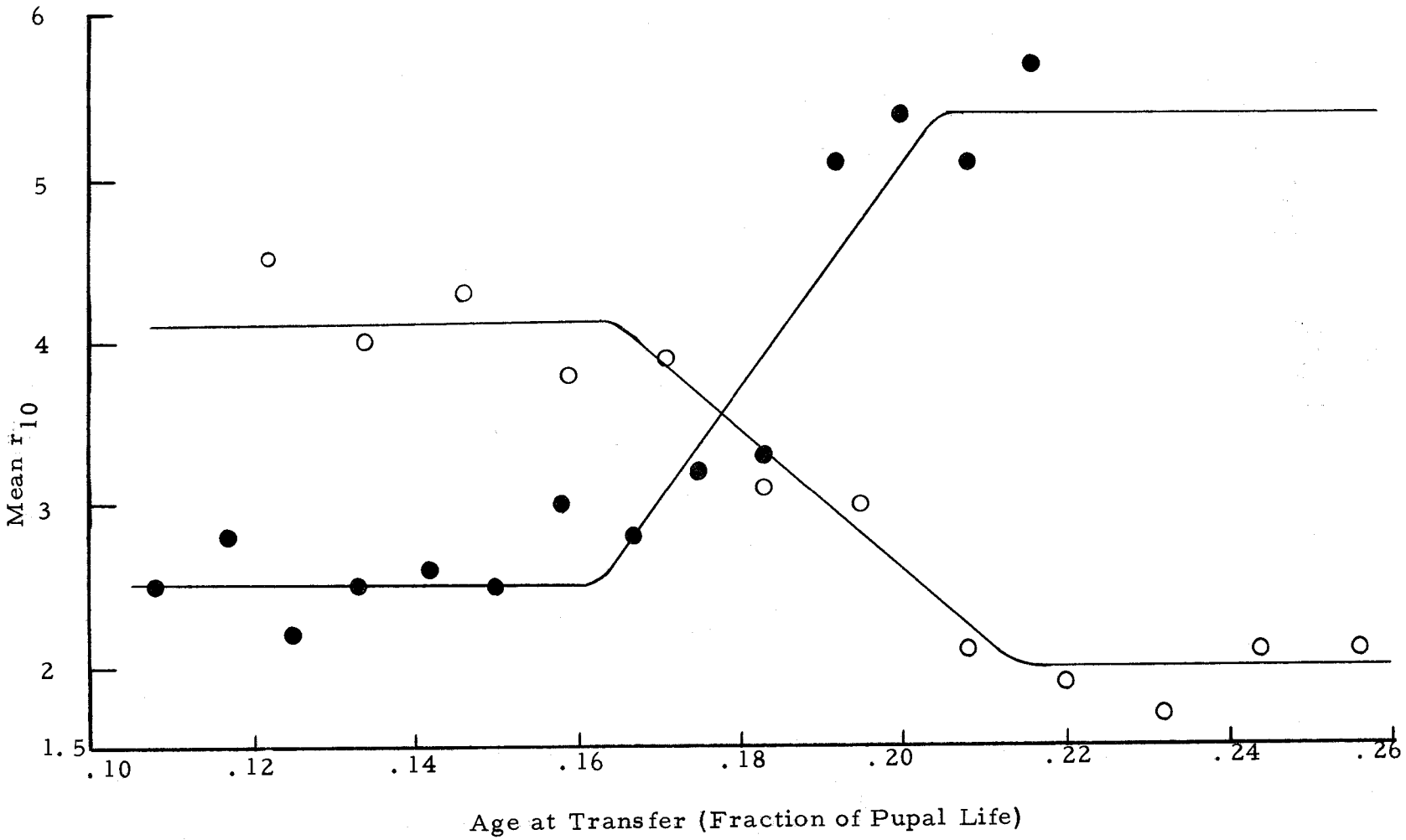

Figure 25. T. E. P. in 5-hi Cy^{+} females at 23° and 28°C. (Open circles - transfers from 28° to 23°C, closed circles - transfers from 23° to 28°C.)

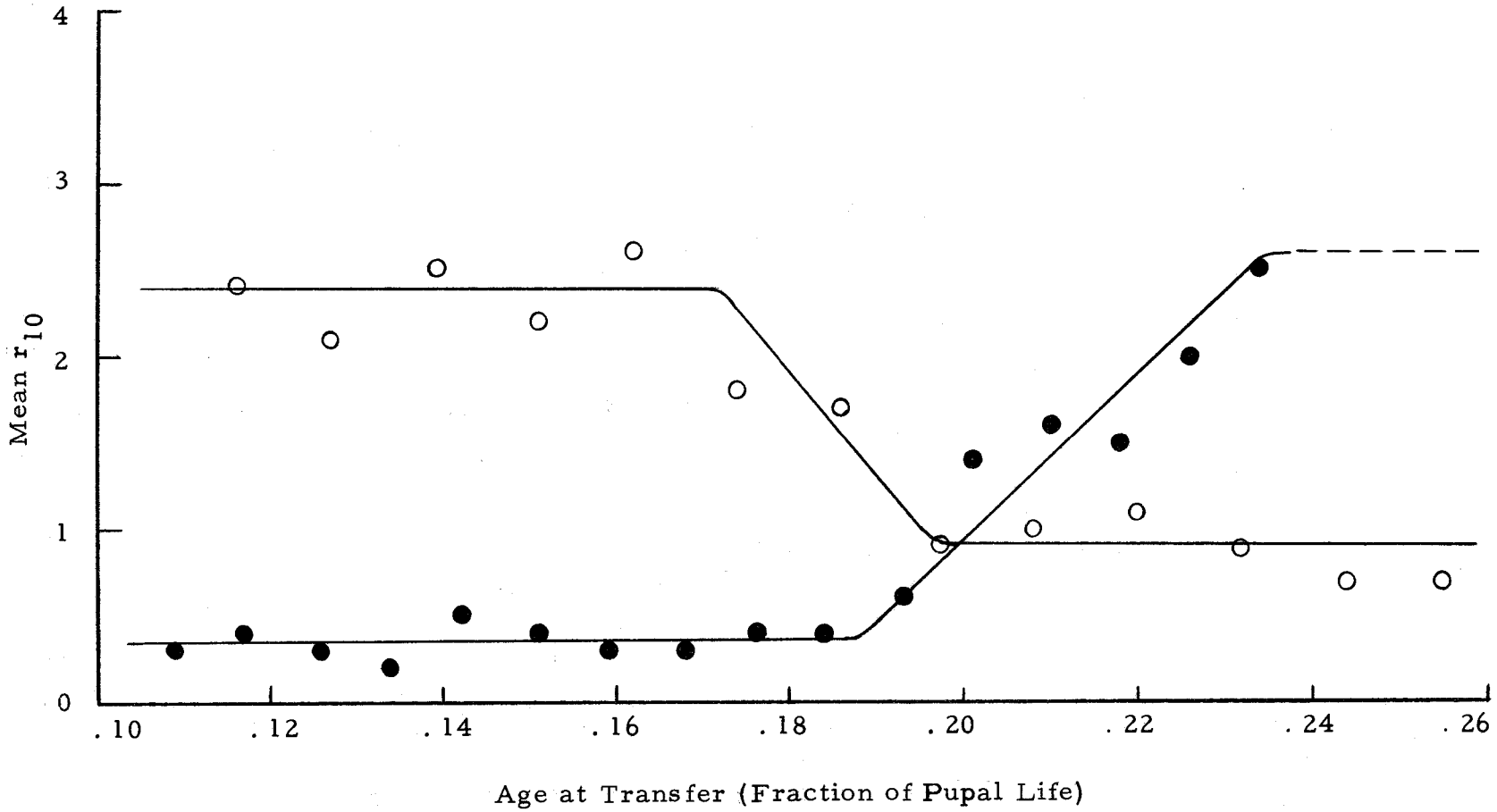

Figure 26. T. E. P. in 5-hi Cy^{+} males at 23° and 28° C. (Open circles - transfers from 28° to 23° C, closed circles - transfers from 23° to 28° C.)

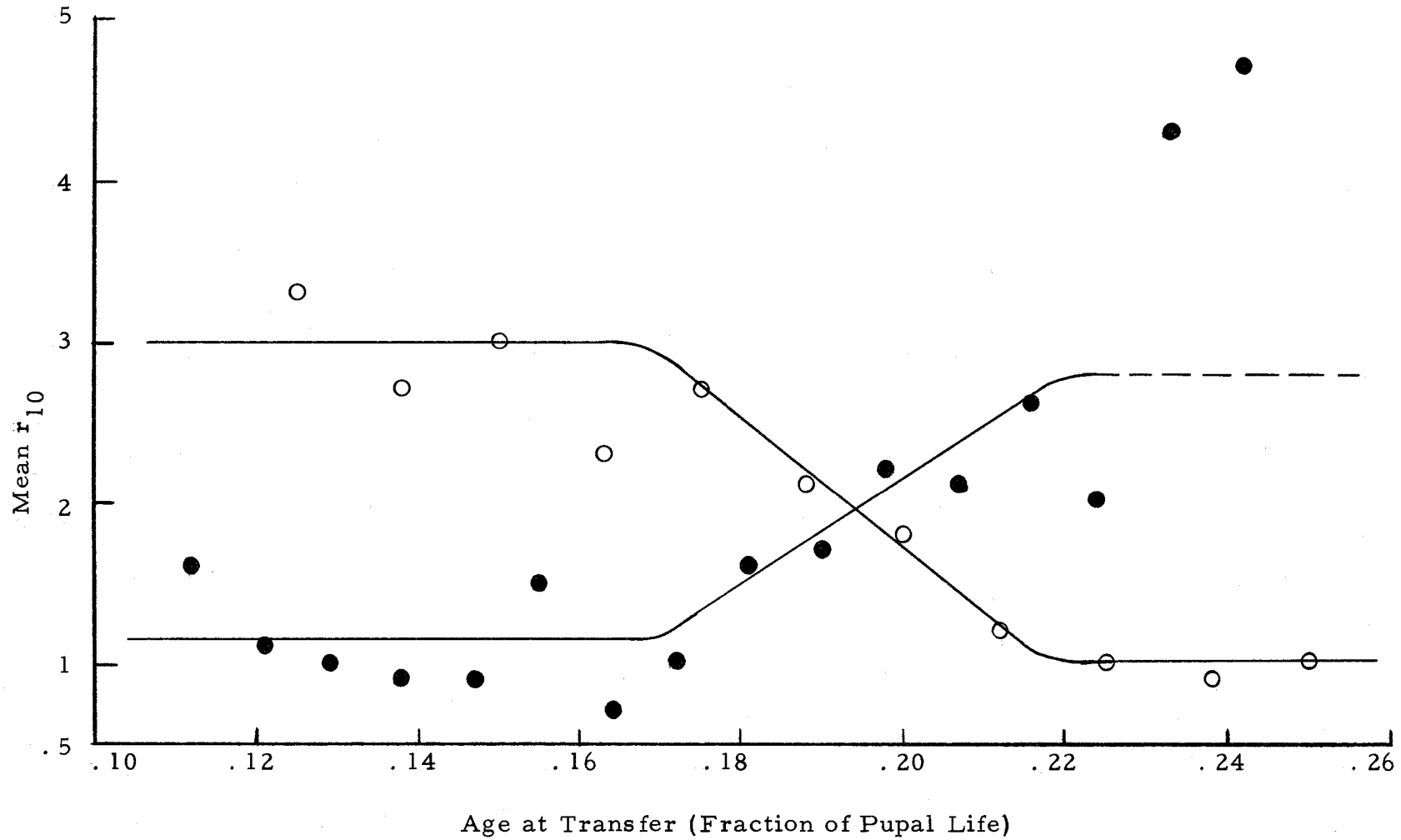

Figure 27. T. E. P. in 5-hi Cy females at 23° and 28° C. (Open circles - transfers from 28° to 23° C, closed circles - transfers from 23° to 28° C.)

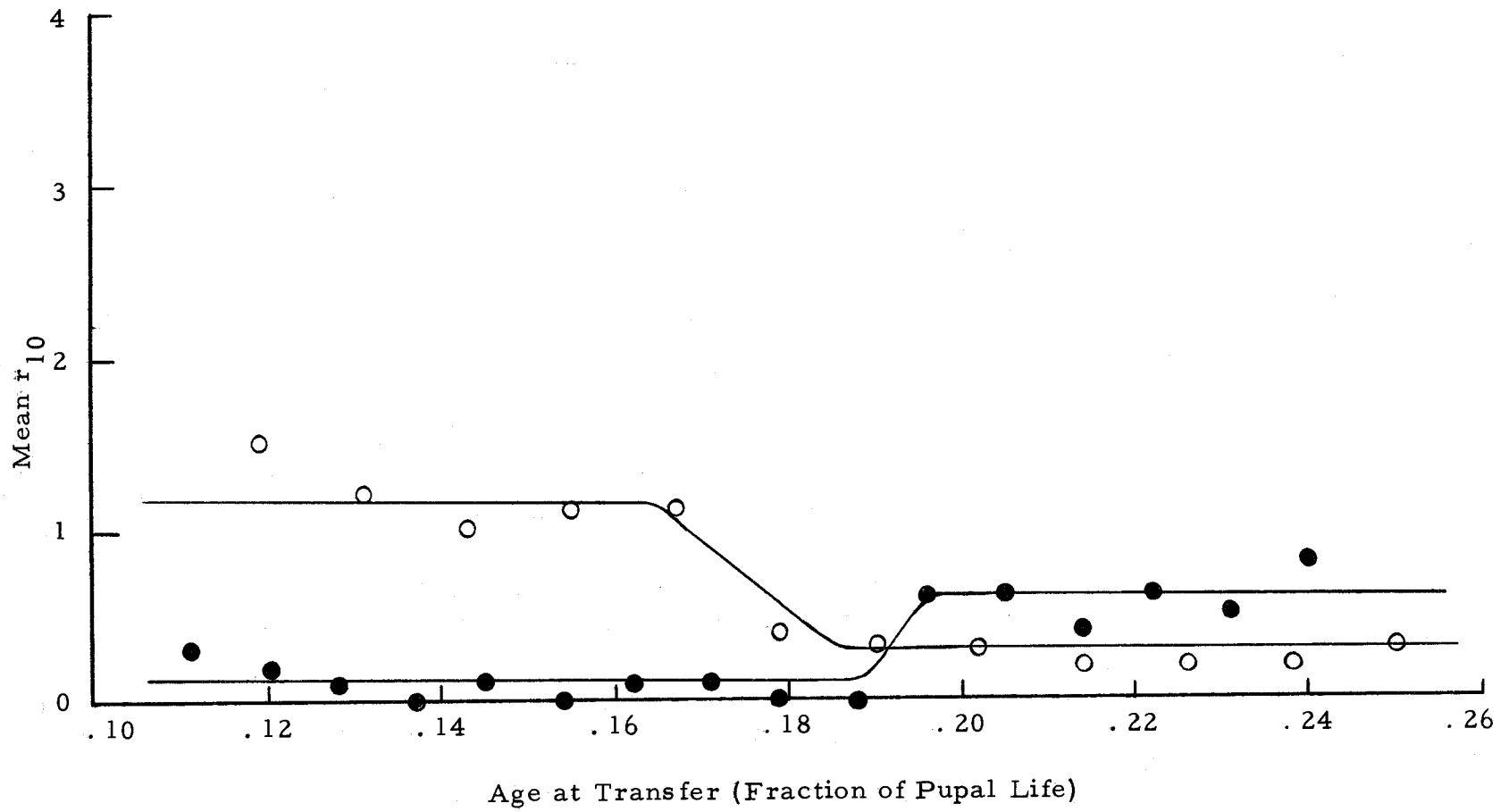

Figure 28. T. E. P. in 5-hi Cy males at 23° and 28° C. (Open circles - transfers from 28° to 23° C, closed circles - transfers from 23° to 28° C.)

In defining T. E. P. 's from these graphs, the plateaus were used as control phenotypes. The alternative controls are the flies grown continuously at one temperature. These alternative controls were often considerably different from the plateaus. The difference suggests an effect due to transferring samples from one temperature to another. A satisfying explanation for this effect (the switching effect) is not available at this time. It may be a result of temperature adaptation as Milkman (1962) pointed out that the temperature response to heat shock in Drosophila pupae is dependent upon its thermal history. In any case, this "switching effect" is quite evident and has also been reported in scute-1 by Child (1935) and in vestigial by Stanley (1931).

In general, the onset of the T. E. P. 's are quite well defined. The end points were not obtained in some cases and in most of the other cases the end points are ambiguous. The starting points in each cvl line at the experimental temperatures as defined by the graphs are summarized in Table 8. The temperatures heading each column is the initial temperature (T_1).

Examination of Table 8 shows that at 28° C, the starting points in the T. E. P. 's of 6-lo and 5-hi were about the same, being at 0. 16 and 0. 17 of pupal life. The T. E. P. of 6-hi at the same temperature started considerably later, at 0. 19 in the females and 0. 20 in the males. At 23° C, both 6-hi and 5-hi have exactly the same starting

Table 8. Temperature-effective periods in 6-hi, 6-lo and 5-hi.

Line	Sex	Time	28°C Starts	28°C Ends	23°C Starts	23°C Ends	18°C Starts	18°C Ends
6-hi	♀	Hours	16	21	16	25		
		Fraction of pupal life	0.19	0.26	0.17	0.22		
	♂	Hours	18	21	21	27		
		Fraction of pupal life	0.20	0.24	0.19	0.22		
6-lo	♀	Hours	13	17			40	44
		Fraction of pupal life	0.16	0.20			0.20	0.22
	♂	Hours	14	18			41	46
		Fraction of pupal life	0.16	0.20			0.19	0.22
5-hi Cy^+	♀	Hours	14	17	20	23		
		Fraction of pupal life	0.17	0.21	0.17	0.19		
	♂	Hours	14	17	23	28		
		Fraction of pupal life	0.16	0.20	0.19	0.23		
5-hi Cy	♀	Hours	14	17	20	23		
		Fraction of pupal life	0.17	0.21	0.17	0.20		
	♂	Hours	14	15	22	23		
		Fraction of pupal life	0.17	0.18	0.19	0.20		

points, 0. 17 in females and 0. 19 in the males. No comparison between lines can be made at 18° C, since the T. E. P. was only determined for 6-lo at this temperature.

Temperature differences in the T. E. P. 's of the cvl lines were found in some cases and not in others. 6-hi females, 5-hi Cy^+ males and 5-hi Cy males seemed to have different starting points at 23° and 28° C and both 6-lo females and males showed a definite difference in starting points at 18° and 28° C. However, that of 6-hi males, 5-hi Cy^+ females and 5-hi Cy females at 23° and 28° C were the same.

Sex differences in the T. E. P. 's of the cvl lines were only apparent at 23° C where females always started at 0. 17 and males at 0. 19 of pupal life. Since the sex difference in peak sensitivity to heat shock (40. 5° C for 20 minutes) is not this large in any strain grown at 23° C (Mohler 1965a, Thompson 1966), the magnitude of the sex difference here is surprising. Starting points of the two sexes in each line were about the same at 28° C and that of the 6-lo line at 18° and 28° C were identical.

From the data on starting points of the T. E. P. 's in the three cvl lines, it seems apparent that differences in T. E. P. 's due to genotype, temperature and sex do occur but only in some cases and under certain conditions.

It should be remarked that the temperature differences in

penetrance and specificity define in each line the same T. E. P. that was defined by expressivity. This shows that the qualitative and quantitative aspects of crossvein development are closely interdependent.

DISCUSSION

The T. E. P. has been determined for each of several characters of Drosophila melanogaster by various workers: Bar (Driver, E. 1926, Driver, O. 1931, Krafka 1920, Luce 1931, Margolis 1934), bar vestigial (Margolis 1934), Notch-deformed (Hillman 1962), scute-1 (Child 1935), tumorous head (Gardner, Turner and Berseth 1960), vestigial (Harnly 1932, Friedland 1941, Stanley 1928, 1931), vestigial-notch (Akita and Nakayama 1954) and vestigial ^{No3}/vestigial^{p} (Cohen and Harnly 1939). It is interesting to note that each T. E. P. occurred only within larval life or embryonic life while the cvl T. E. P. is clearly within pupal life. Some of the workers were concerned with the quantitative aspects of development and used the T. E. P. to explain the phenotypic expression of a character at different temperatures. The assumption was made that the quantitative aspects of a character can be explained by the rate of reaction in processes leading to the formation of the character and the duration of these processes, which is the T. E. P. However, these two factors may or may not be independent of each other. Child (1935) pointed out that such explanations are not justified unless we know the exact rate of the developmental processes in question. He argued for bristles affected by scute-1 that the duration and the position in development of the T. E. P. varied from fly to fly. Therefore, in order to imply

developmental rates from the T. E. P., it is necessary to have very accurate data. Such accuracy may require extensive experiments and detailed statistical analysis. No workers before Child have taken this into consideration. Since the duration of the T. E. P. in each cvl line studied here is not accurately measured, expressivity variation in terms of quantitative development is excluded. The quantitative picture is confused further by the "switching effect".

Specificity also changes with temperature (Tables 3 and 4). This effect was quite obvious when working with the 6-lo females in which the L4 expression was predominant at 18° C and the L5 expression was predominant at 28° C. The frequencies of L4 and L5 interruptions in each control sample of 6-lo females, calculated among the total number of wings, is shown in Table 9. These data reveal that the temperature effect on specificity in 6-lo females is a consequence of a differential expression for the two parts of the crossvein. With a decrease in temperature, the frequency of L4 interruptions consistently increases but the frequency of L5 interruptions remains fairly constant. In phenocopy studies with a different cvl-6b strain, Mohler (1965a), found that the spontaneous expression was predominantly at L5 but that the phenocopy expression was at L4. The heat shock effect on specificity was a result of an increase in the frequency of L4 interruptions and, at the same time, a decrease in the frequency of L5 interruptions. He interpreted this as two opposing responses to

heat shock differentially expressed at the two ends of the crossvein. Though there is no evidence of opposing responses to temperature changes within the physiological range the results are of interest as they also point out the differentiation of the two parts of the crossvein.

Table 9. Frequency of L4 and L5 interruptions in 6-lo females.

Experiment No.	Temp.	Percent L4*	Percent L5*
1	18	62	2. 3
	23	17. 4	14. 5
	28	0. 9	13
2	18	45	7. 2
	28	0	6. 7
3	18	62	9. 7
	28	0. 5	5. 2

*Calculated from the total number of wings in each sample.

SUMMARY

1. The durations of pupal development in three crossvein-like (cvl) lines, 6-hi, 6-lo and 5-hi, are estimated from their mean emergence times at the three experimental temperatures.

2. The temperature coefficient for the rate of early morphological development at 23° and 28° C is determined in 6-hi and found to be similar to that for the overall rate of development at the same temperatures.

3. Temperature differences in phenotypes are found in all three cvl lines.

4. The temperature-effective period (T. E. P.) of each cvl line occurs within the interval between one-eighth and one-quarter of pupal life.

5. The onset of the T. E. P. in each line is fairly well defined. The end points are missing in some cases and are ambiguous in others. Complications are caused by the "switching effect".

6. Temperature, genotypic and sex effects on the T. E. P. are suggested in some cases but not evident in others.

7. Theoretical considerations are given to the T. E. P. in terms of quantitative and qualitative expression of phenotypes.

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Pupal Developmental Time of 6-hi and 6-lo

		♀					♂				
Line	Temp.	Hrs	n	Σ	%	S.E.	Hrs	n	Σ	%	S.E.
6-hi	18°C	199-5/6	19	22	86.4	7.3	213-1/2	16	23	69.6	9.6
		198-5/6	17	22	77.3	8.9	212-1/2	20	24	83.3	7.6
		197-5/6	15	20	75	9.7	211-1/2	20	27	74.1	8.4
		196-5/6	15	29	51.7	9.3	210-1/2	10	23	43.5	10.0
		195-5/6	8	19	42.1	11.0	209-1/2	10	22	45.5	11.0
		194-5/6	11	28	39.3	9.2	208-1/2	7	18	38.9	11.0
		193-5/6	2	28	7.1	4.9	207-1/2	4	17	23.5	10.0
		192-5/6	2	20	10	6.7	206-1/2	1	20	5	4.9
		191-5/6	0	23	0	0	205-1/2	2	23	8.7	5.9
		190-5/6	0	17	0	0	204-1/2	0	19	0	0
6-hi	23°C	116-5/12	17	26	80	8.0	123-5/6	10	19	66.7	10.0
		115-5/12	20	25	65.3	9.3	122-5/6	14	21	52.6	11.0
		114-5/12	13	18	72.2	10.0	121-5/6	10	24	41.7	10.0
		113-5/12	3	22	13.6	7.3	120-5/6	2	28	7.1	4.9
		112-5/12	1	19	5.3	5.1	119-5/6	0	26	0	0
6-hi	28°C	86-1/6	23	24	95.8	4.1	89-2/3	15	19	79	9.3
		85-1/6	24	27	89	6.0	88-2/3	13	21	62	10.0
		84-1/6	26	26	100	0	87-2/3	10	21	47.6	11.0
		83-1/6	14	24	58.3	10.0	86-2/3	5	29	17.2	7.0
		82-1/6	9	27	33.3	9.1	85-2/3	0	22	0	0
6-lo	18°C	200-3/4	31	37	83.7	6.1	212-3/4	16	17	94	5.8
		199-3/4	23	32	72	7.9	211-3/4	24	25	96	3.9
		198-3/4	16	30	53.3	9.1	210-3/4	23	24	96	4.0
		197-3/4	13	31	42	8.9	209-3/4	24	27	89	6.8
		196-3/4	6	22	27.3	9.5	208-3/4	21	27	77.8	8.0
		195-3/4	2	31	6.5	4.4	207-3/4	14	19	73.7	10.0
		194-3/4	3	21	1.4	2.6	206-3/4	11	30	40	8.9
		193-3/4	0	18	0	0	205-3/4	13	35	37	8.2
		192-3/4	0	22	0	0	204-3/4	4	30	13.3	6.2
		191-3/4	0	33	0	0	203-3/4	0	35	0	0
6-lo	23°C	118-5/6	26	26	100	0	125-1/12	19	19	100	0
		117-5/6	36	36	100	0	124-1/12	19	19	100	0
		116-5/6	27	29	93	4.7	123-1/12	20	26	77	8.3
		115-5/6	26	26	100	0	122-1/12	22	29	76	7.9
		114-5/6	16	19	84.2	8.4	121-1/12	5	21	23.8	9.3
		113-5/6	17	27	63	9.3	120-1/12	2	26	7.7	5.2
		112-5/6	13	27	48.2	9.6	119-1/12	0	25	0	0
		111-5/6	8	23	34.8	9.9	118-1/12	0	29	0	0
		110-5/6	1	26	3.8	3.8	117-1/12	0	25	0	0
6-lo	28°C	89-7/12	27	28	96.5	3.5	92-1/2	23	23	100	0
		88-7/12	26	27	96.3	3.6	91-1/2	20	22	91	6.1
		87-7/12	33	34	97	2.9	90-1/2	15	15	100	0
		86-7/12	24	26	92.3	5.2	89-1/2	22	24	91.6	5.7
		85-7/12	20	22	91	6.1	88-1/2	19	26	73	8.7
		84-7/12	19	28	68	8.8	87-1/2	7	20	35	11.0
		83-7/12	14	24	58.4	10.0	86-1/2	6	22	27.2	9.5
		82-7/12	5	25	20	8.0	85-1/2	0	21	0	0

Pupal Developmental Time of 5-hi

		♀					♂				
Line	Temp.	Hrs	n	Σ	%	S.E.	Hrs	n	Σ	%	S.E.
Cy^+	23°C	122-1/6	29	29	100	0	122-1/6	28	30	93.5	4.5
		121-1/6	22	22	100	0	121-1/6	26	34	71.5	7.7
		120-1/6	17	21	81	8.6	120-1/6	25	36	69.5	7.7
		119-1/6	12	35	34.3	8.0	119-1/6	13	26	50	9.8
		118-1/6	9	34	26.4	7.6	118-1/6	7	36	19.5	6.6
		117-1/6	6	26	23	8.3	117-1/6	0	28	0	0
		116-1/6	4	34	11.8	5.5	116-1/6	0	34	0	0
Cy^+	28°C	87-5/6	46	46	100	0	90	36	37	97.5	2.5
		86-5/6	34	35	97.3	2.7	89	45	47	95.7	3.0
		85-5/6	34	35	97.3	2.7	88	34	40	85	5.6
		84-5/6	39	41	95.2	3.3	87	34	44	77.3	6.3
		83-5/6	37	39	95	3.5	86	14	32	43.8	8.8
		83-5/6	31	49	63.3	5.9	85	9	35	25.7	7.4
		81-5/6	20	47	42.6	7.2	84	0	33	0	0
		80-5/6	5	34	14.7	6.1	83	0	35	0	0
Cy	23°C	121-1/4	17	18	94	5.6	121-1/4	25	26	96	3.8
		120-1/4	21	22	95	4.6	120-1/4	31	32	97	3.0
		119-1/4	24	25	96	3.9	119-1/4	22	26	85	7.0
		118-1/4	16	19	84	8.4	118-1/4	14	21	74	9.6
		117-1/4	15	21	71	9.9	117-1/4	14	15	67	10.0
		116-1/4	18	25	72	9.0	116-1/4	1	28	4	3.7
		115-1/4	5	15	33	12.0	115-1/5	0	19	0	0
Cy	28°C	87-5/6	9	9	100	0	87-5/6	10	10	100	0
		86-5/6	9	9	100	0	86-5/6	8	8	100	0
		85-5/6	16	16	100	0	85-5/6	15	16	93.7	6.0
		84-5/6	10	10	100	0	84-5/6	8	10	80	13.0
		83-5/6	11	11	100	0	83-5/6	9	20	45	11.0
		82-5/6	13	14	92.8	6.9	82-5/6	2	14	14.3	9.4
		81-5/6	14	14	100	0	81-5/6	0	14	0	0
		80-5/6	20	23	83.5	7.7	80-5/6	0	19	0	0

Determination of Temperature-Effective Periods in 6-hi and 6-lo

	T_1 to T_2 *					T_2 to T_1 *				
	Hrs	♀		♂		Hrs	♀		♂	
Line	in T_1	n	$\bar{r}_{10}$	n	$\bar{r}_{10}$	in T_2	n	$\bar{r}_{10}$	n	$\bar{r}_{10}$
6-hi	29	41	9.2	41	8.2	21	39	7.1	28	5.8
	28	39	9.1	26	7.8	20	39	7.7	48	6.6
	27	17	9.0	16	8.1	19	36	8.2	32	7.3
	26	16	9.2	14	6.8	18	45	8.3	27	8.3
	25	39	8.8	26	6.1	17	74	8.4	59	8.2
	24	44	8.3	46	5.6	16	53	9.0	34	8.3
	23	30	8.0	62	5.0	15	48	8.9	47	8.3
	22	45	7.3	48	4.8	14	51	8.9	43	8.2
	21	48	7.5	42	5.1	13	53	9.1	44	8.8
	20	22	7.5	17	4.5	12	25	9.2	25	8.4
	19	32	6.9	22	4.1	11	38	9.1	23	9.1
	18	38	7.2	38	4.8	10	41	9.1	38	8.4
	17	40	6.8	32	4.4					
	16	36	7.0	48	4.4					
	15	41	6.9	38	4.5					
	14	40	7.5	39	4.9					
6-lo	53	64	3.2	41	1.8	28	48	0.3	46	0
	52	55	2.8	51	2.1	27	45	0.1	46	0
	51	49	3.6	54	2.3	26	61	0.2	33	0
	50	52	3.3	58	2.8	25	52	0.3	44	0.1
	49	54	3.4	53	2.3	24	62	0.3	32	0
	48	39	3.5	52	2.2	23	64	0.2	31	0
	47	56	3.5	39	1.8	22	51	0.3	29	0.1
	46	52	3.7	43	2.1	21	51	0	34	0
	45	75	2.6	56	1.5	20	57	0.2	50	0.1
	44	68	3.3	44	1.5	19	63	0.2	41	0.1
	43	58	2.6	52	0.7	18	57	0.2	33	0.1
	42	43	2.2	56	0.6	17	49	0.4	49	0.7
	41	53	1.1	53	0.3	16	45	1.1	49	1.0
	40	54	0.6	48	0.1	15	53	2.3	43	1.5
	39	48	0.7	50	0.1	14	60	2.4	46	2.5
	38	58	0.7	42	0.1	13	59	3.1	45	2.7
	37	56	0.5	58	0					
	36	52	0.4	46	0					
	35	54	0.4	47	0					
	34	56	0.3	38	0					

*

For 6-hi: $T_1 = 23^{\circ}C$, $T_2 = 28^{\circ}C$.

For 6-lo: $T_1 = 18^{\circ}C$, $T_2 = 28^{\circ}C$.

Determination of Temperature-Effective Periods in 5-hi

Line	23°C to 28°C: Hrs in 23°C	♀ n	♀ $\bar{r}_{10}$	♂ n	♂ $\bar{r}_{10}$	28°C to 23°C: Hrs in 28°C	♀ n	♀ $\bar{r}_{10}$	♂ n	♂ $\bar{r}_{10}$
Cy$^+$	28	52	8.3	84	2.5	23	-	-	51	0,1
	27	58	7.2	62	2.0	22	-	-	42	0.7
	26	62	5.7	60	1.5	21	61	2.1	47	0.7
	25	54	5.0	62	1.6	20	74	2.1	61	0.9
	24	53	5.4	59	1.4	19	59	1.7	26	1.1
	23	62	5.1	49	0.6	18	64	1.9	40	1.0
	22	57	3.3	72	0.4	17	65	2.1	35	0.9
	21	73	3.2	66	0.4	16	75	3.0	40	1.7
	20	73	2.8	68	0.3	15	73	3.1	36	1.8
	19	67	3.0	63	0.3	14	67	3.9	62	2.6
	18	66	2.5	69	0.4	13	55	3.8	63	2.2
	17	58	2.6	73	0.5	12	44	4.3	55	2.5
	16	72	2.5	73	0.2	11	44	4.0	58	2.1
	15	70	2.2	85	0.3	10	58	4.5	45	2.4
	14	74	2.8	63	0.4					
	13	26	2.5	56	0.3					
Cy	28	27	4.7	28	0.8	23	-	-	40	0.1
	27	37	4.3	31	0.5	22	-	-	37	0.1
	26	23	2.0	39	0.6	21	44	0.9	47	0.3
	25	32	2.6	38	0.4	20	45	1.0	38	0.2
	24	23	2.1	34	0.6	19	43	0.9	44	0.2
	23	28	2.2	23	0.6	18	37	1.0	31	0.2
	22	25	1.7	14	0	17	46	1.2	45	0.3
	21	21	1.6	24	0	16	50	1.8	28	0.3
	20	39	1.0	22	0.1	15	48	2.1	40	0.4
	19	26	0.7	21	0.1	14	38	2.7	38	1.1
	18	24	1.5	36	0	13	43	2.3	48	1.1
	17	31	0.9	29	0.1	12	53	3.0	47	1.0
	16	20	0.9	29	0	11	42	2.7	55	1.2
	15	28	1.0	21	0.1	10	33	3.3	50	1.5
	14	31	1.1	33	0.2					
	13	10	1.6	8	0.3					