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Final Report

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Title: Nutritional Requirements and Breeding Behavior of *Perognathus*

I. Protein Requirements:

A. In continuation of our studies of the reasons why casein cannot replace soy protein in our semi-synthetic diet (see previous semi-annual report) we have found that the casein we used contained SO_2 (Joslyn, M. A. and J. Leichter, Thiamine instability in experimental wet diets containing commercial casein with sulfur dioxide, J. Nutr. 96: 1, 1968, 89-93) and suspected that this may lead to the destruction of thiamine and maybe other nutrients. The use of SO_2-free protein did, however, not improve its adequacy for P.Pe. or P.Lo. The problem, therefore, needs further investigation.

B. We have tried still another protein to substitute for soy protein. 4 Pe. and 4 Lo. were fed a diet similar to gly-7 but egg albumin (22%) was substituted for soy protein. The Pe. are alive but not looking too well after 4 months, while all Lo. died within 3 weeks. We think that this differential response is more a function of body size than of species difference, analogous to the longer survival of Kangaroo rats on Bell's diet compared to Pe.

C. The study of the effects of methionine have not shown interpretable results (Table I). The addition of methionine at a level of 1.25% to the diet composed of natural food stuffs (sunflower and millet seeds) has no deleterious effect on either P.Pe. or P.Lo. Lower levels of methionine in a synthetic diet containing soy protein, or casein, however, appear to be toxic to both species. Although the number of animals used were insufficient it appears as if P.Pe. may be more susceptible to methionine toxicity while P.Lo. may be more susceptible to deleterious effects of a high protein content regardless of the amount of methionine.

II. Response of Kangaroo Rats (*Dipodomys merriam*):

We have shown previously (see previous semi-annual report) that this desert rodent has similar dietary requirements as does *Perognathus*. They do well on semi-synthetic diet and cannot tolerate the Bell's diet. We have now tried the supplementation of this lethal diet with carrots and found that this modification leads to an adequate diet for these animals as it does for *Perognathus*. A group of Kangaroo rats is doing well after 8 months on this diet.

III. Adequacy of the semi-synthetic diet for maintenance of adult *Per. Longimembris*: We have previously reported that the semi-synthetic diet is adequate for P.Pe. in terms of maintenance of organ weight, carcass composition and hematological parameters. (The possibility that the diet could not fully maintain normal hematological parameters was shown to be due to technical difficulties. On retesting neither the semi-synthetic diet nor Bell's plus carrots diet showed a deleterious effect on the hematocrit or blood count). We have now tested P.Lo. for the same parameters. Four diet groups were studied: Mixed seeds for 2 months (1), mixed seeds for 1 year (2), semi-purified diet for 1 year (3), and Bell's + carrots for 6 months (4) (for diet composition see previous semi-annual reports). The results show (Table II) that body weights and organ weights were the same in all groups, a finding similar to that in P.Pe. The carcass composition of the animals fed seed diets (1 and 2) were similar to P.Pe. fed the same diet, although P.Lo. tended to have a higher proportion of fat (and, of course, less water) than P.Pe.

The males fed the seed diet had much less fat than those fed either the semi-synthetic or the Bell's + carrot diet (Table III). This effect of diet is also reflected in the combined data (Table II), although the differences were not statistically significant because of the large individual variation in the females. An increase in body fat has not been oberved in male P.Pe. fed the same semi-synthetic diet, when compared with seed-fed animals. This may be due to the fact that the group of P.Pe. tested had been on this diet for only 2 months, while the present group of P.Lo. had been on the diet for 1 year.

As in the case of P.Pe., the hematological data were similar for P.Lo. fed seeds, taken to be their "natural" diet and those fed the semi-synthetic diet for the same length of time (Table IV; Group 2 and 3 respectively). The same is probably true for those fed the Bell's plus carrot diet. (One out of the 3 animals tested was obviously out of line.)

A comparison of animals in Group 1 which had been in the laboratory for only 2 months with the other groups with residence ranging from 1-2 years shows a curious increase in MCHC with length of residence in the laboratory. (Group 4 animals had been in the laboratory for 1-2 years before placing on the Bell's + carrot diet.) Whether these data are of significance is, however, doubtful.

The apparent decrease of the hematocrit in Groups 2, 3, and 4 compared to Group 1 is probably an artifact. Blood was drawn into syringes wet with heparin solution for Groups 2, 3, and 4 while they were first dried for Group 1. Since only 150 µlt blood was obtained a dilution with 10-15 µlt would account for the lower hematocrits.

IV. Breeding Behavior:

We have previously reported that the induction of estrus in P.Pe. by "screen-pairing" (semi-annual report Aug.-Dec. 1967) seems to be influenced by the photoperiod. We are now reporting preliminary results of experiments which were designed to investigate the effects of a constant long or short photoperiod on spontaneous and induced estrus.

1. Effect of constant 16-hour photoperiod:

Five Parous females screen-paired and under long photoperiod have cycled regularly since Dec. 15, 1967 (Figure I.). The periods between estrus which was 10-15 days has gradually lengthened (since about middle of May, e.g. after 5 months) and is now (Sept. 1, e.g. after 9 months) 25-28 days.

This seems to indicate a slow exhaustion of the regulatory hormonal mechanisms. Another group of mice who had been under the same photoperiod but singly caged were screen-caged July 26 (e.g. after 7 months on 16-hour day but not stimulated). They are cycling now with a period of 10-12 days. (They had not been checked for spontaneous estrus during the period in single cages.) This indicates that a long photoperiod alone does not lead to exhaustion of the hormonal mechanism or not at the same rate as when combined with stimulation.

2. Effect of constant 10-hour photoperiod:

Of four screen-paired females under a short photoperiod since Feb. 1 (e.g. 7 months) two have been cycling at the normal period of 12-14 days, lengthening since mid-July (e.g. after 5 months) to 17-19 days, one stopped on July 18 and one began cycling in mid-July with a 15-20 day period (e.g. after 4 months screen-pairing) (Fig. 2) This indicates that a short photoperiod *per se* does not prevent stimulated estrus behavior, but that it may lead to an "out-of-phase" cycling. Of four singly-caged females one has cycled at 13-15 days since March 28 (e.g. 5 months), one stopped cycling in the middle of May (e.g. after 2 months) and two have begun cycling at the end of Aug. (e.g. after 5 months). This strengthens the impression that a short photoperiod leads to estrus behavior out-of-phase with natural season. It also indicates that under our conditions some females show persistent, spontaneous estrus under a very short photoperiod.

3. Effect of changing photoperiod:

This group of animals was housed in a room where the artificial light was supposed to be synchronized with sunrise and sunset so that the photoperiod lengthened in spring and decreased in fall. Of four screen-paired females, two have cycled since the middle of Jan. (e.g. 9 months). (Fig. 3) The other two started cycling in mid-April at 13.5 hours/day (e.g. after 4 months screen-pairing without cycling). Of four singly-caged females, one started cycling at a photoperiod of 11 hours, two at 12 hours and one at 14 hours. They are all cycling now (12-hour photoperiod) at somewhat irregular, but lengthened periods between estrus. The results of this experiment are difficult to interpret. We have discovered that due to technical difficulties the photoperiod in this room may not, in fact, have been what they were designed to be but may have varied at random from time to time. They do, however, confirm that some of these females will cycle for a least 9 consecutive months and that the period tends to increase after 5 months.

Summary:

1. The adequacy of the semi-purified diet was tested on P.Lo. It was found to maintain body weight, organ weights and hematological parameters at the level found in a group of animals fed a mixed seed diet. Body fat of male P.Lo., but not of females, appears to be increased in response to the synthetic diet.

2. Further study of the protein requirements of pocket mice has shown that:

   a) Egg albumin cannot replace soy protein in the semi-purified diet.

   b) P.Lo. seems to have a better tolerance for high methionine levels than does P.Pe.

3. Study of the influence of photoperiod on estrus behavior of P.Pe. has shown that neither a long photoperiod (16 hours) nor a short photoperiod (10 hours) supresses spontaneous or induced estrus for at least 7-9 months. It does, however, produce a lengthening of the time between estrus and permits onset and cessation of estrus irrespective of natural season. The persistence of both spontaneous and induced estrus under a 10-hour photoperiod is a puzzling observation. We are now investigating whether other clues, for instance olfactory stimulation by material transferred on the cotton gloves used to handle the animals, may be overriding the normal effects of a short photoperiod.

Table I. Effects of methionine and level of protein on survival of P.Pe. and P.Lo.

Diet*	% Methionine	Survival			
		P.Pe. alive after:	(months)	P.Lo. alive after:	(months)
30% soy	0.40	3/5	7	1/4	1.5
30% soy	0.90	0/4	1	0/4	1
22% soy	0.37	4/4	>12	4/4	>12
22% soy	0.87	0/4	3	2/2	10
22% casein	0.72	0/4	1.5	0/4	1.5
SMD	0.75	4/4	>12	4/4	>12
SMD	1.25	3/4	8	2/2	8

*For diet composition see semi-annual report January-June, 1967.

TABLE II. Body and organ weights and carcass composition of *Perognathus longimembris* fed different diets.

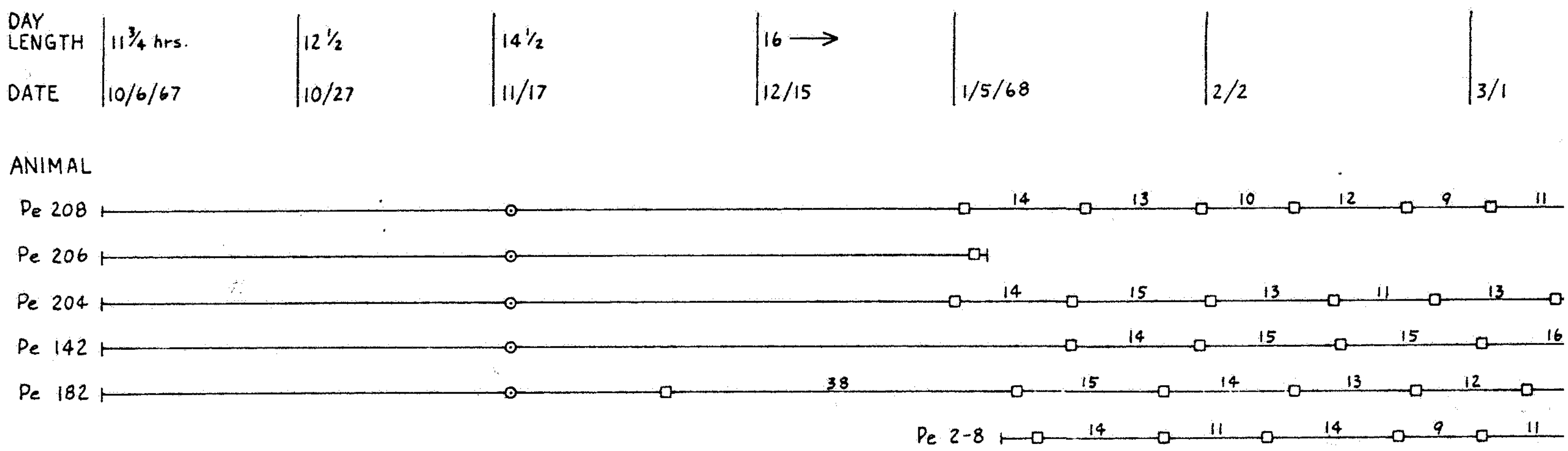
	Animal number	Body wt. at autopsy g	Carcass wt. g	Moisture	Fat	Protein	Protein[1]	Liver	Heart	Kidney	Lung
				g/100 g fresh carcass				g/100 g body wt.			
Group 1 Mixed Seeds 2 months[3]	512♀	8.2	4.19	54.2	22.3	18.9	80.7	4.4	0.7	1.3	0.7
	514♀	11.2	7.16	38.2	42.6	15.2	78.8	3.6	0.6	1.0	0.5
	519♂	8.2	4.43	59.1	17.8	18.8	81.2	4.1	0.6	1.2	0.6
	521♂	9.1	5.10	58.7	17.8	19.0	80.6	3.2	0.5	1.2	0.6
	Mean	9.2	5.22	52.6	25.1	18.0	80.3	3.8	0.6	1.2	0.6
	SE[2]			4.9	5.9	0.9	0.5				
Group 2 Mixed Seeds 1 year	170♀	10.7	6.49	44.9	33.6	17.0	79.1	4.6	0.6	1.2	0.6
	174♀	7.1	3.15	59.7	11.9	22.0	77.7	3.7	0.6	1.1	0.7
	121♂	9.9	5.67	56.2	21.0	18.2	79.6	3.4	0.6	0.9	0.7
	205♂	9.0	5.44	58.3	19.2	17.8	78.9	3.3	0.5	1.1	0.6
	Mean	9.2	5.19	54.8	21.4	18.8	78.8	3.8	0.6	1.1	0.6
	SE			3.4	5.4	1.1	0.4				
Group 3 Semipurified Diet 1 year	178♀	8.7	5.00	41.9	36.0	17.2	77.9	3.3	0.7	1.1	0.7
	186♀	10.2	6.00	39.0	39.8	17.6	83.5	3.4	0.6	1.0	0.9
	15♂	11.1	6.77	40.7	36.9	18.1	80.6	3.5	0.6	1.1	0.5
	253♂	10.2	6.18	35.6	47.5	13.6	80.5	3.6	0.6	1.1	0.7
	41♂	8.9	4.81	46.6	30.5	19.3	83.4	3.8	0.7	1.2	0.6
	202♀	8.7	4.40	49.6	27.3	18.5	79.9	3.8	0.6	1.3	0.6
	209♂	8.7	4.20	57.6	18.2	19.4	80.1	3.6	0.5	1.3	0.6
	Mean	9.5	5.74	44.4	33.7	17.7	80.8	3.6	0.6	1.2	0.7
	SE			2.8	3.6	0.7	0.8				
Group 4 Bell's plus carrots 6 months	259♂	9.6	5.33	46.1	31.0	19.5	85.3	3.9	0.7	1.0	0.7
	261♂	10.9	6.04	50.4	28.3	17.7	82.5	2.9	0.6	1.2	0.4
	263♂	9.9	5.98	41.5	39.1	16.1	83.4	3.4	0.5	1.0	0.5
	Mean	10.1	5.78	46.0	32.8	17.8	83.7	3.4	0.6	1.1	0.5
	SE			2.6	3.2	1.0	0.8				

[1]Grams/100 grams of dry, fat-free carcass.

[2]Standard errors were not calculated for body, carcass or organ weights because these were very constant and obviously not statistically different.

[3]For diet composition see previous semi-annual report.

Figure 1. ESTRUS BEHAVIO



Legend:

Screen-paired

Singly-caged

Estrus observed

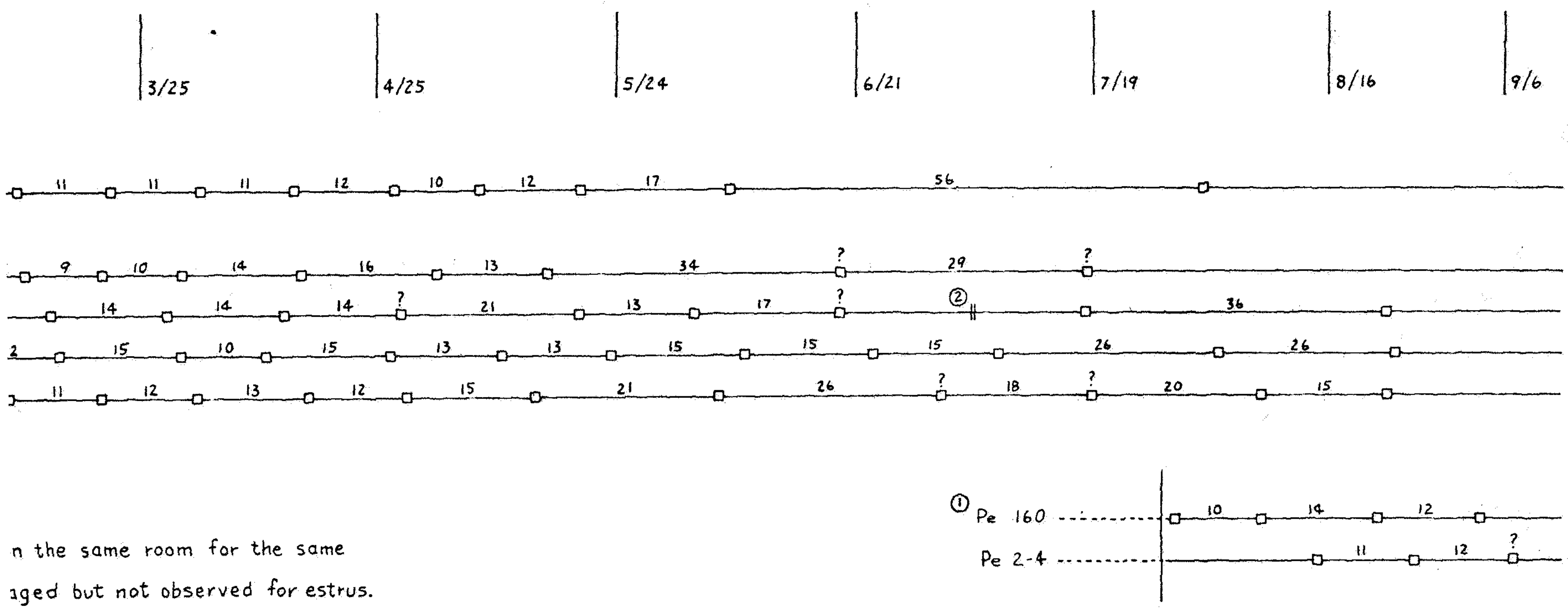
Days between estrus

Injection with 6 IU PMS
(estrus expected after 48-72 hours)

(1) These animals had been
length of time, singly-

(2) Animal died; replaced

OF P.PE. UNDER A LONG PHOTOPERIOD



n the same room for the same
aged but not observed for estrus.

Pe 504.

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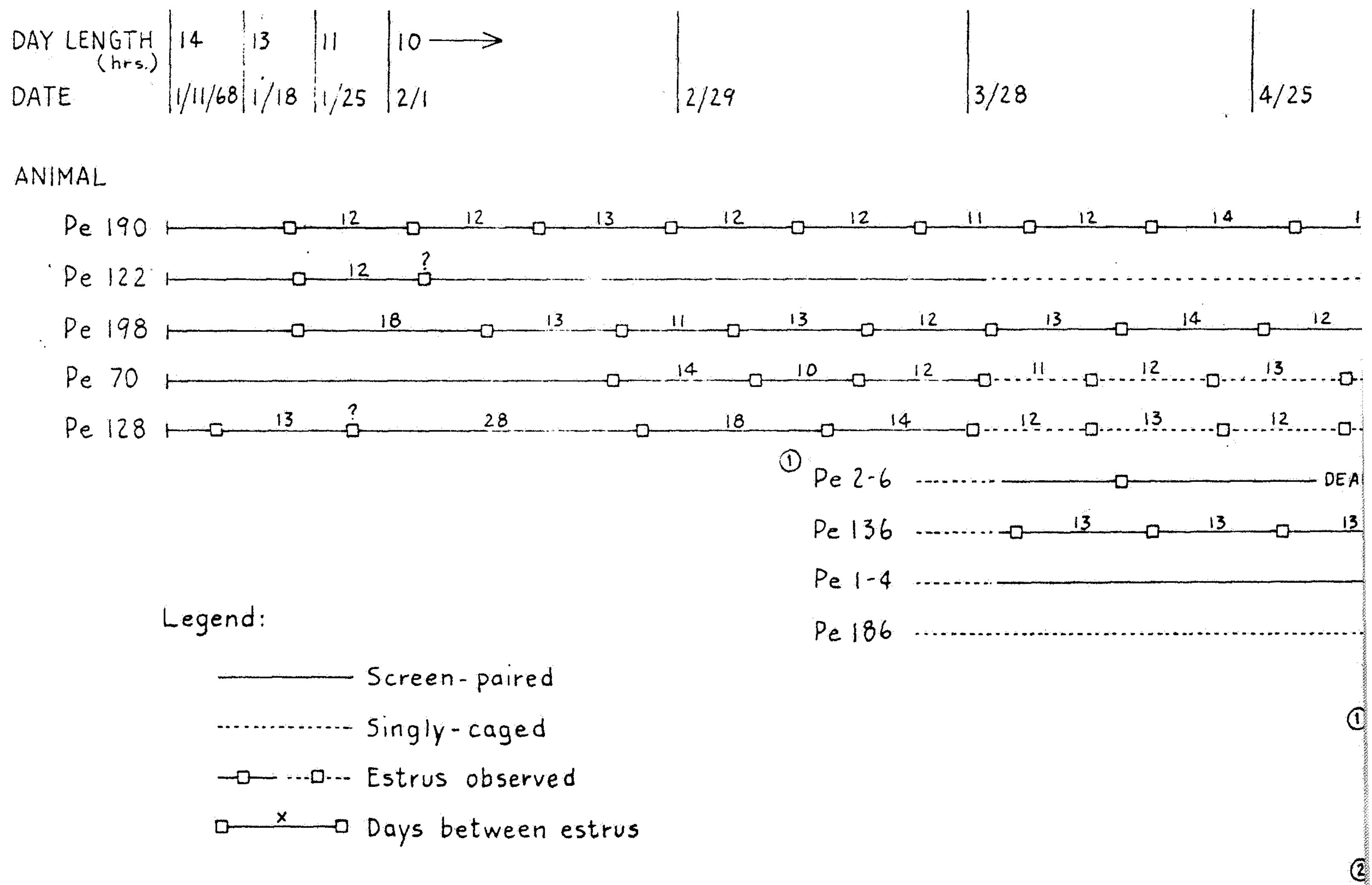
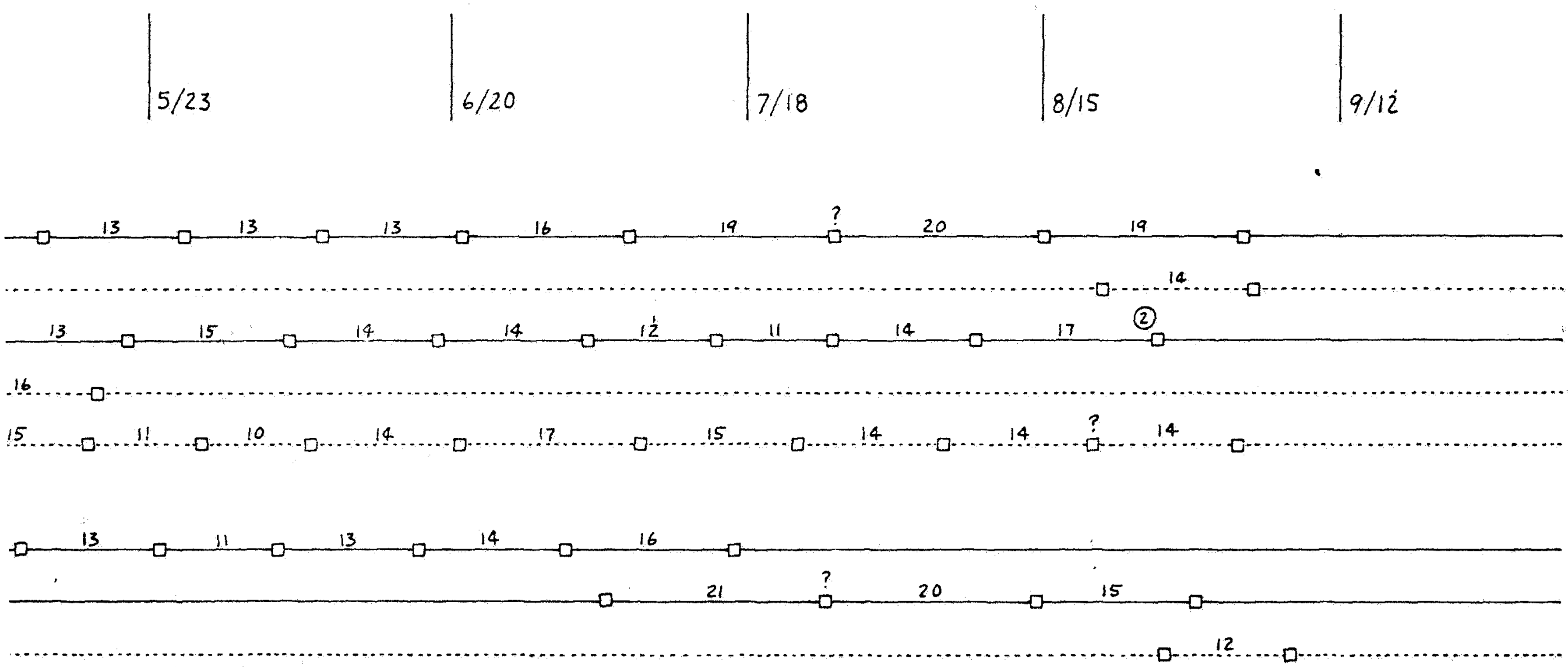
Figure 2. ESTRUS BEHAVIOR



F P.PE. UNDER A SHORT PHOTOPERIOD

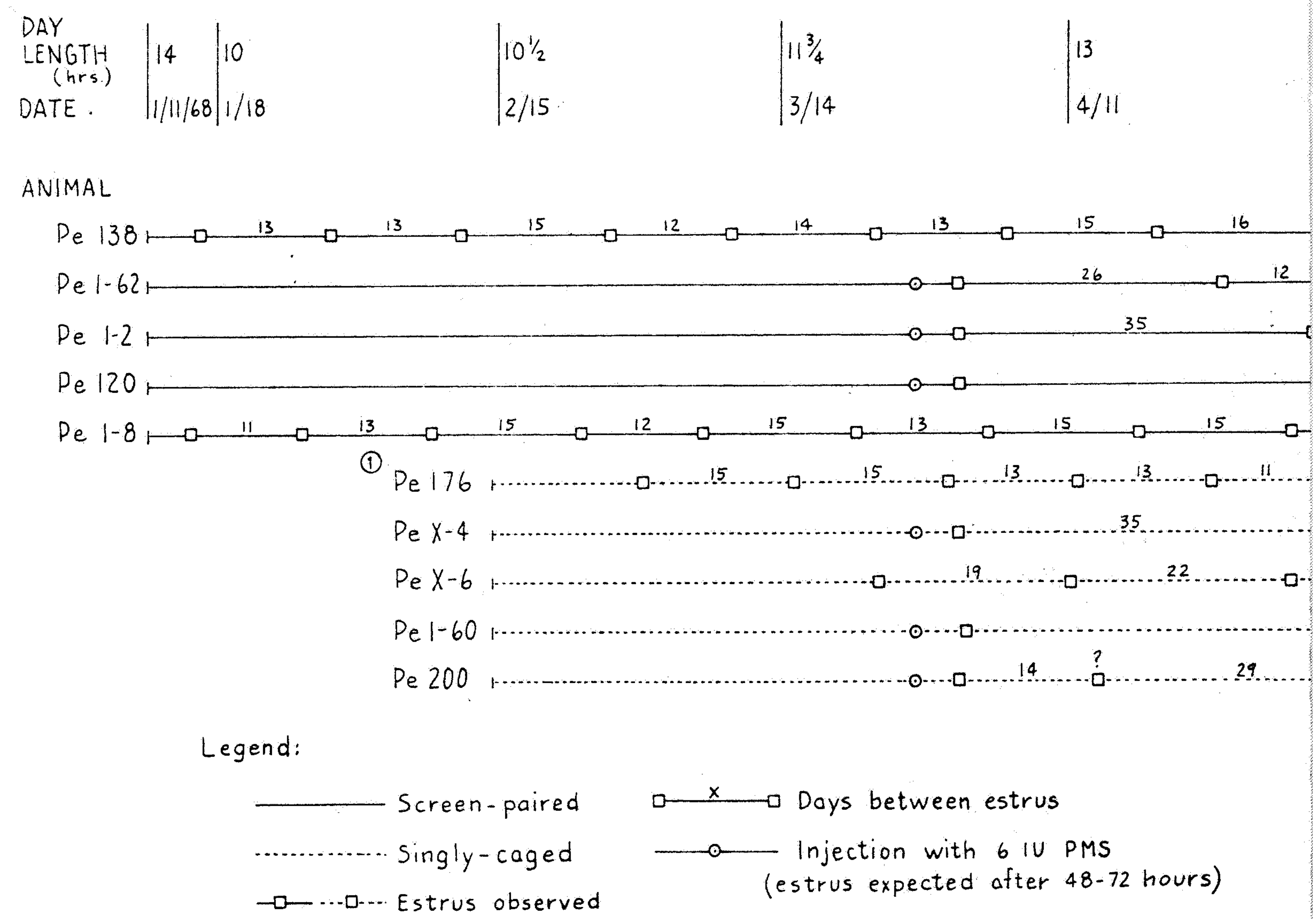


ese animals had been in the same room for the same
ngth of time, singly-caged but not observed for estrus.

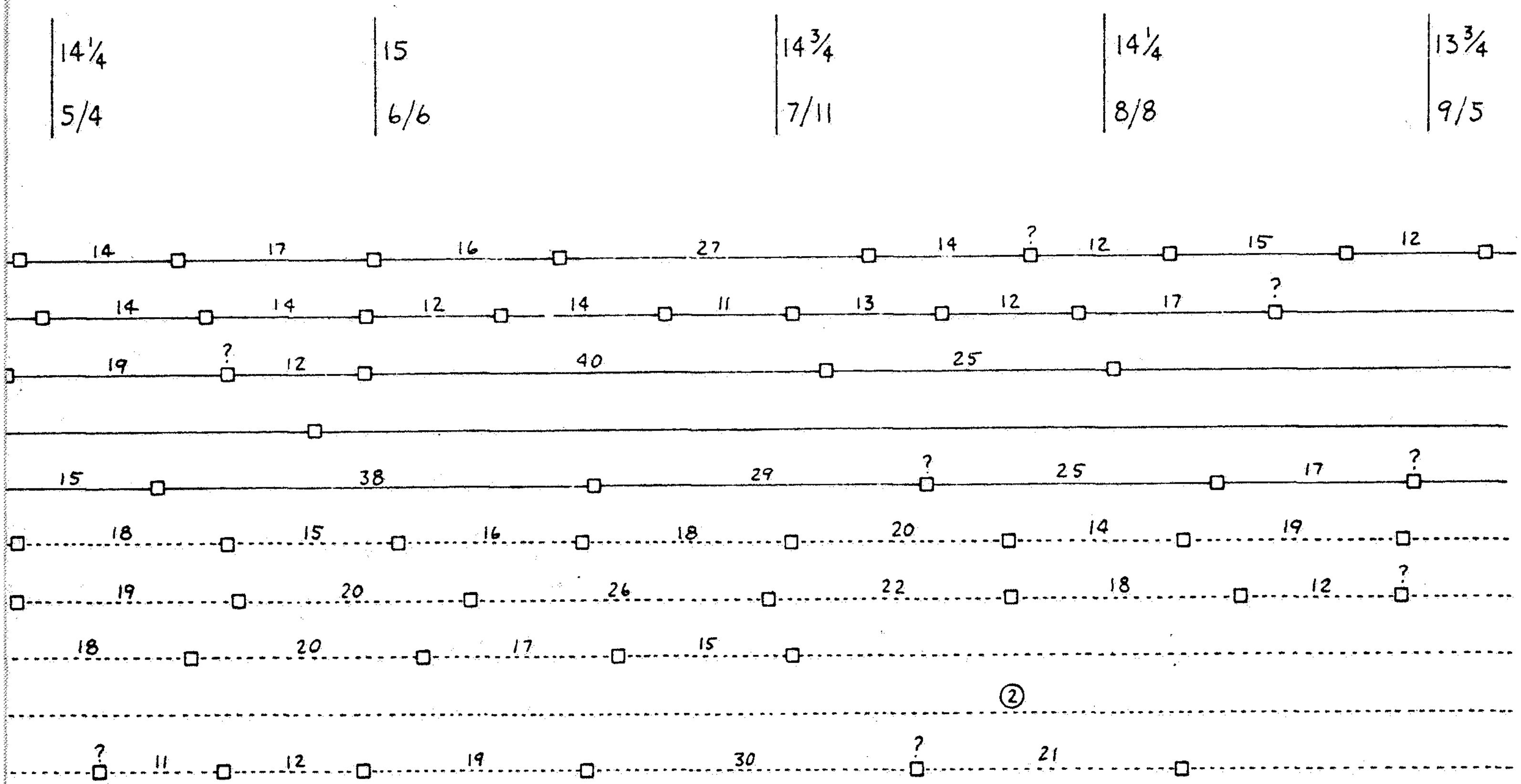
:cidentally mated?

- / -

Figure 3. ESTRUS BEHAVIOR OF P. PE



UNDER A CHANGING PHOTOPERIOD



① These animals had been in the same room, singly-caged but not observed for estrus.

② Replaced with Pe 126

Table III. Sex difference in body fat of P.Lo. fed different diets (g/100 g fresh carcass)

	Mixed seeds I (2 months)		Mixed seeds II (1 year)		Semipurified diet III (1 year)		Bell's + Carrots IV (6 months)	
	Male	Female	Male	Female	Male	Female	Male	Female
	17.8	22.3	21.0	33.6	36.9	36.0	31.0	--
	17.8	42.6	19.2	11.9*	47.5	39.8	28.3	--
					30.5	27.3	39.1	
					18.2			
Average	17.8	32.4	20.1	22.7	33.3	34.4	32.8	--

*This animal weighed only 7 g and was possibly sick.

Table IV. Hematological data of *Perognathus longimembris* fed different diets.

	Animal number	Hematocrit %	Hemoglobin %	R.B.C./mm^3 ($X\ 10^6$)	W.B.C./mm^3 ($X10^3$)	MCV[1]	MCH[2]	MCHC[3]
Group 1 Mixed Seeds 2 months	505♂, 515♂	50.0	18.0	12.2	2.90			
	510♀, 516♀	47.0	16.8	13.7	----			
	502♀	48.0	15.4	----	----			
	522♀	49.0	15.7	12.6	1.85			
	Mean	48.5	16.5	12.8	2.38	38.0	12.9	34.0
Group 2 Mixed Seeds 1 year	170♀	45.0	17.1	13.6	3.36			
	174♀	43.0	14.5	14.1	0.85			
	121♂	43.0	15.5	12.3	0.95			
	205♂	47.0	16.4	11.4	3.15			
	Mean	44.5	15.9	12.8	2.08	34.8	12.4	35.7
Group 3 Semipurified diet 1 year	178♀	46.0	16.4	14.1	0.65			
	41♂	42.0	16.1	9.6	1.40			
	202♀	43.0	16.4	11.7	1.10			
	209♂	41.0	14.7	10.7	0.90			
	Mean	43.0	15.9	11.5	1.01	37.4	13.8	36.9
Group 4 Bell's plus carrots 6 months	259♂	44.0	17.1	10.5	1.65			
	261♂	30.0	11.0	7.2	2.20			
	263♂	42.0	16.2	12.0	3.90			
	Mean	37.2	14.8	9.9	2.58	37.6	15.0	39.7
	Mean[4]	43.0	16.6	11.5	2.14	37.4	14.6	38.6

[1]MCV = mean corpuscular volume. $\frac{\text{Hematocrit}}{\text{RBC}} \times 10 = \mu^3$

[2]MCH = mean corpuscular hemoglobin. $\frac{\text{Hemaglobin}}{\text{RBC}} \times 10 = \mu\mu g$

[3]MCHC = mean corpuscular hemoglobin concentration. $\frac{\text{Hemoglobin}}{\text{Hematocrit}} \times 100 = \%$

[4]Mean values without animal #261 who is obviously out-of-line.