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Energy Budgets of Animals:
Behavioral and Ecological Implications

Progress Report

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Abstract

Proposed Technical Scope

Lizard growth and reproduction experiments will involve completion of egg hatching requirements (temperature and soil moisture), metabolism measurements of developing eggs, determination of factors controlling growth rate and continuation of long-term longevity studies of lizards growing seven times faster than field rates. Deer mouse growth and reproduction experiments with different levels of food and water deprivation and other variables will continue. Physiological parameters necessary for a general predictive heat and mass transfer model will be gathered if equipment can be purchased.

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Report of progress on Contract EY-76-S-02-2270.A 002

Introduction

ERDA support is helping us develop general microclimate and population dynamics models for selected ectotherms and endotherms. The demonstration of the feasibility of developing predictive models for climate and microenvironment effects on populations of animals will make it possible to truly quantitatively assess environmental impacts of a variety of man's activities that are energy related. Even the magnitude and cost of revegetation efforts to reclaim mining or construction sites should be affected by these general models. We have recently demonstrated the generality of our microclimate and ectotherm models for successfully predicting activity patterns and food requirements for all ages of a tropical lizard. The original tests of the models were in the Mojave desert and used the desert iguana (Porter et al., 1973). Now we have shown (Porter and James, 1977; James and Porter, 1977) that the models also work for tropical environments and tropical lizards. The required parameters are relatively easily obtained and are listed in Porter and James (1977).

Summary of results

With ERDA support we are now extending the predictive capacity of the models beyond maintenance (survival) requirements to environmental effects on requirements for growth and reproduction in selected ectotherms and endotherms. In this report we describe results this past year on

1. Ectotherms (desert iguana)

- a. First successful breeding of this animal ever in the lab. This is now our third year.
- b. We have shown that a supposedly "k" selected animal (long lived, slow growing, one clutch per year) can with proper environmental exposure become an "r" selected animal (short lived (?), fast growing, up to three clutches per year)
- c. We have demonstrated a growth rate six to seven times the growth rate observed in the field.
- d. We have demonstrated the attainment of sexual maturity in 7 1/2 months instead of 6-7 years in the field.
- e. We have demonstrated that as the females get larger they put less, not more of their resources into egg production, counter to present theory.
- f. We have shown that egg development rates can vary from 42-69 days depending on temperature.
- g. We have shown that developing lizard eggs can tolerate soil moistures lower than the wilting point of domestic plants.
- h. We have preliminary evidence that suggests that the developing eggs "dehydrate" during development, which makes them able to absorb water from soils which might be drying in midsummer as the end of incubation nears.

2. Endotherms (deer mouse)

- a. We have shown that the role of food and water in controlling the capacity to reproduce shifts with levels of

available food and water. There are heirarchical effects such that available food is what has the first effect on reproductive capacity when reductions from ad lib consumption occur. Then water available becomes critical at still lower percentages of ad lib consumption. At this point availability of fresh green materials and a nest shelter to reduce evaporative water loss appear to become very important. The population as a whole responds to various levels of water deprivation in apparently sigmoidal fashion. There is a rapid drop to about 60% survival of young at about 90% ad lib water consumption. This survival level then declines slowly to about 40% survival at about 40% ad lib water consumption. At 20% ad lib water consumption there is total reproductive failure. Perhaps most interesting is that even down to 40% ad lib water, there are some mothers who can bring through a complete litter to weaning. Thus there appears to be a great resilience in the population to water deprivation, if available food does not drop below 90% ad lib consumption when not pregnant.

3. "Cactus" experiments

- a. There appear to be critical densities of spines at which convective enhancement increases up to as much as 70% higher than "cacti" with spine densities slightly lower or higher.

Part I Ectotherms (Desert iguana)

Colony Status as of January 1, 1977

Adult ♀♀	51	42 field captured	9 1975 "hatchlings"
Adult ♂♂	31	26 field captured	5 1975 "hatchlings"
Juveniles	<u>43</u>	--	43 1976 hatchlings
	125		

-- All 1976 hatchlings (43) are expected to reach maturity by August, 1977. This projection is based on current growth rates and growth rates of 1975 hatchlings. All 1975 hatchlings (14) reached adult size in less than 1 year.

Egg Production

34 clutches produced 160 eggs

18 bad clutches (clutches for which the control and all experimental eggs failed to hatch)

16 good clutches (clutches for which the control hatched)

Bad eggs (80) were the result of the female failing to construct a burrow, collapsed burrows, or apparently infertile egg production.

The good eggs (75) produced 48 hatchlings from the control and experimental incubation conditions. Mortality was due to developmental abnormality (3) and thermal injury resulting from hatchlings becoming stuck to tape beneath lights (2). This design flaw has been eliminated from all cages.

53% of the ♀♀ (27/51) produced clutches. Some ♀♀ produced multiple clutches. Of the 1975 hatchling ♀♀, 67% (6/9) produced clutches. These ♀♀ mated with 2 1975 hatchling ♂♂.

Water Potential and Temperature Tolerance Experiments

The control condition was arbitrarily chosen as 36°C and 0.5 bar water potential. These conditions were successful in preliminary experiments conducted in 1975. All incubation experiments were steady-state temperature and water potential experiments. One egg from each clutch was selected as a control for the entire clutch. The remaining eggs were incubated at experimental conditions (see below).

The results of 1976 incubation experiments are summarized below. The 2 numbers in the left hand corner of a block are respectively the number of eggs incubated (upper figure) and the number of eggs that hatched (lower figure). The percentage of eggs hatching at a given experimental condition is also indicated in the block.

Water Potential (bars)

Temp (°C)	-0.5	-1	-5	-10
25		1 0 0% ⁺	3 0 0% ⁺	4 0 0% ⁺
30		6 5 83%	2 1 50%	
36	16 16 100%	4 4 100%	10 9 90%	9 8 89%
40		7 3 43%*	6 2 33%*	6 4 67%* ← # eggs started ← # eggs hatching

*abnormal; severe developmental abnormality of spine and central nervous system

⁺well developed embryos that failed to hatch

The upper and lower temperature tolerance appears to be about 40°C and 25°C respectively. The lower water potential (i.e. greater negatively) limit is above -10 bars. Subsequent analysis of data indicates a fully hydrated egg will have a water potential of about -12 to -13 bars. Future incubation experiments will be directed toward refining the temperature data and delimiting the upper water potential tolerance (in the -15 to -20 bar range).

Relationships Between Female Size, Clutch Size and Egg Weight

There was no correlation between the mass or snout-vent length of a ♀ and the number of eggs per clutch. The mass of a female is the mass after ovaposition.

All eggs were collected within 12 hr. of laying. The eggs were held for 24 hrs at the control conditions in order to standardize weights between clutches. The distribution of egg weights and clutch size are given in fig. 1.

Individual egg weight decreases as clutch size increases (Fig. 2 & 3). One way Anova statistical tests indicate the mean egg weight per clutch decreases significantly ($P < .05$) with increasing clutch size. These data were for all eggs ($N=141$) regardless of the condition of the clutch (i.e. both good and bad clutches).

Conflicting results obtain if only egg weights from 100% successful clutches of a given size are considered. These are clutches in which no egg failed to hatch in the control or experimental group. There were 49 such eggs ranging from 3.3 to 5.3 g.

The average egg weight per clutch of size N (4,5,6) was not significantly different by oneway Anova or t-test. The average clutch weight (total weight of all eggs in a clutch) per clutch of size N (4,5,6) did not differ significantly by oneway Anova. Between group comparison by t-test indicated NSD between average clutch weights of 4 & 5 and 5 & 6 egg clutches. There was a SD between average clutch weight of 4 & 6 egg clutches ($P < .05$). The within group variance was large. I suspect the large variance and small sample size combined to produce the above conflicting results. This problem should be resolved with a larger sample size.

"Reproductive effort" is inversely related to ♀ body weight. Reproductive effort is defined as the clutch weight/body weight ratio. This is a simplistic measure of "effort" because it does not account for many costs associated with maintenance, egg production, food gathering, etc. A rigorous measure of reproductive effort must show the proportion of the total resources of a ♀ that are allocated to producing a clutch. There exists no complete data set (that I know of) to make this type of measurement, hence the simple definition of reproductive effort is used here. Reproductive effort as a function of body weight for all clutches and for good clutches only are given in figs. 4 & 5 respectively.

Incubation: the influence of temperature and water potential

The relationships between temperature, water potential and incubation time are summarized in Table 1. The table is a block diagram of between group t-tests where S and NSD are respectively significant and no significant difference at $P < .05$ level. The mean incubation time in days of a comparison row is given in the "X (days)" column (ex. 69 days is the mean incubation time for eggs incubated at 30°C and -1 bar water potential).

Table 1 indicates some general trends, but I do not want to draw firm conclusions until more data are available for intermediate temperatures and water potentials in excess of -10 bars. Further caution should be taken in interpreting the 40° data: all hatchlings exhibited morphological abnormalities and the sample size is very small. Data from 38°C incubation will be completed in spring 1977. These data will help interpretation of the 40°C data.

The difference in mean incubation time between the 30°C and 36°C groups is on the order of 25 days over a 6°C temperature range. A much shorter difference in incubation time over the 4°C range between 36°C and 40°C is apparent. The difference is on the order of 2 days, much shorter than expected. It is of interest to note that adult lizards exhibit temperature independent metabolic rates (O_2 consumption) between 36° and 40°C.

Incubation time, at a given temperature, decreases as water potential decreases (i.e. becomes larger negatively). This trend is clear at 40° (means 48.0-44.4 days). The 36° data for -5 and -10 bars also follows this trend. Additional data to be collected in 1977 will clarify the trend noted above.

--- HISTOGRAM C36 EGG WT. FOR ALL EGGS

MIDDLE OF INTERVAL	NUMBER OF OBSERVATIONS	
2.5	6	*****
3.0	8	*****
3.5	17	*****
4.0	34	*****
4.5	42	*****
5.0	23	*****
5.5	7	*****
6.0	2	**
6.5	1	*
7.0	1	*

-- HISTOGRAM C45 EGG WT. Good clutches

MIDDLE OF INTERVAL	NUMBER OF OBSERVATIONS	
2.4	1	*
2.8	1	*
3.2	2	**
3.6	6	*****
4.0	16	*****
4.4	20	*****
4.8	19	*****
5.2	6	*****

-- HISTOGRAM C47 NUMBER OF EGGS IN CLUTCH, ALL CLUTCHES
EACH * REPRESENTS 2 OBSERVATIONS

MIDDLE OF INTERVAL	NUMBER OF OBSERVATIONS	
3.	9	*****
4.	34	*****
5.	60	*****
6.	38	*****

-- HISTOGRAM C5 NUMBER OF EGGS IN GOOD CLUTCHES

MIDDLE OF INTERVAL	NUMBER OF OBSERVATIONS	
4.	19	*****
5.	30	*****
6.	26	*****

Fig. 1.

-- PLOT EGG WEIGHT, ALL EGGS (G) C36 VS NUMBER OF EGGS IN CLUTCH C47

* , one observation
 + , ≥ 10 observations

Fig. 2

-- PLOT EGG WEIGHT, GOOD CLUTCHES (G) C43 VS NUMBER OF EGGS IN CLUTCH C5

C43

5.60+

-

- 2

- 2

-

4.80+

- 3

- 4

- 2

- 3

4.60+

- 2

-

-

-

3.20+

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2.40+

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1.60+

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4.00

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C5

4.50

5.50

6.50

Fig. 3.

All Clutches $N=30$

[Clutch wt./Body wt. ratio] * 100 vs ♀ Body Wt. (g)

"Reproductive Effort"

-- PLOT CLUTCH WT. / BODY WEIGHT RATIO C48 VS FEMALE BODY WEIGHT (g) C49

Good Clutches

91.0+

84.0+

77.0+

70.0+

63.0+

56.0+

49.0+

42.0+

35.0+

28.0+

$N=15$

$r=.755$

12

Fig. 5

		30	36			40			36	40	$\bar{X}$ (days)
		1	1	5	10	1	5	10	All	All	
30	1	—	⁺ ₋ S	⁺ ₋ S	⁺ ₋ S	⁺ ₋ S	⁺ ₋ S	⁺ ₋ S			69.0
36	1		—	⁻ ₊ S	⁻ ₊ NSD	⁻ ₊ S	⁻ ₊ S	⁻ ₊ S			43.0
	5			—	⁺ ₋ S	⁻ ₊ S	⁻ ₊ NSD	⁻ ₊ NSD			45.3
	10				—	⁻ ₊ S	⁻ ₊ S	⁻ ₊ NSD			43.5
40	1					—	⁺ ₋ S	⁺ ₋ S			48.0
	5						—	⁺ ₋ S			46.0
	10							—			44.4
Control		⁻ ₊ S	⁺ ₋ S	⁺ ₋ S	⁺ ₋ NSD	⁻ ₊ S	⁻ ₊ S	⁺ ₋ NSD	⁺ ₋ NSD	⁻ ₊ NSD	44.9

Average incubation time at T and Ψ

* t test NSD, ANOVA SD (barely)

⁺, ⁻ within a box indicates mean incubation time > or < the mean of comparison, rows on left, column on right

Table 1

Part II Endotherms (deer mice)

This past year has been principally an exploration or screening for variables that substantially affect growth and reproduction in deer mice. We already know which parameters affect the mass and heat energy balance of the animals, which in turn affect maintenance, growth and reproductive requirements in different environments. However, those variables that are not so easily quantifiable, such as number of previous litters, age of the mother, photoperiod, and so on, must also be identified. The magnitude of their effect in the context of the physical variables that affect growth and reproductive potential must be established. The marriage of empirically determined variables and their effects with predictive mechanistic equations for mass and energy balance equations is in its infancy. We have been using an iterative fractional factorial design to screen variables. The technique is described in the appendix. Ideally, one would like to be able to run full factorial designs with replications. Practically, time, energy and money constraints require an iterative fractional approach to permit resolution of first main effects, then two factor interactions, then if necessary three or more factor interactions if it becomes obvious that higher order effects are large.

The design and results of the first experiment on deer mice are illustrated in Tables 1, 2, 3 and 4.

TABLE 1 EXPERIMENT #1

P. maniculatis

VARIABLE NUMBER		LEVEL		CENTER REPLICATE
		(—)	(+)	
1	SPROUTS	NONE	NORMAL	$\frac{1}{2}$ NORMAL
2	WEIGH FREQ.	1/DAY	1/3 DAYS	1/2 DAYS
3	NEST BOX	NO	YES	NO ROOF
4	REMOVE YOUNG TO 2	NO	YES	REMOVE TO 3
5	♂ PRESENCE	YES	NO	$\frac{1}{2}$ - 1 HOUR ♂ OUT
6	EXERCISE WHEEL	LOCKED	UNLOCKED	LOCKED EVERY OTHER DAY
7	FOOD	80%	ADLIB	90%
8	WATER	80%	ADLIB	90%

Table 2
 Experiment #1
 2^{8-4} _{IV} design; trying 1 replicate/run;
 1 center replicate/block

21 day
 young

BLOCK #	RUN #	VARIABLE								AVERAGE WEIGHT
		1	2	3	4	5	6	7	8	
1	2	+	-	-	-	-	+	+	+	9.0
	10	-	+	+	+	+	-	-	-	0.
2	5	-	-	+	+	-	-	+	+	8.75
	13	+	+	-	-	+	+	-	-	5.10
3	6	+	-	+	-	+	+	-	+	0.
	14	-	+	-	+	-	-	+	-	8.5
4	1	-	-	-	+	+	-	-	+	0.
	9	+	+	+	-	-	+	+	-	7.3
5	7	-	+	+	-	-	-	-	+	5.18
	15	+	-	-	+	+	+	+	-	6.8
6	4	+	+	-	+	-	+	-	+	0.
	12	-	-	+	-	+	-	+	-	4.78
7	3	-	+	-	-	+	-	+	+	5.62
	11	+	-	+	+	-	+	-	-	0.
8	8	+	+	+	+	+	+	+	+	10.2
	16	-	-	-	-	-	-	-	-	4.87
		<u>.7</u>	<u>7.7</u>	<u>-3.8</u>	<u>-7.6</u>	<u>-11.1</u>	<u>.7</u>	<u>45.8</u>	<u>1.4</u>	

(.7/8)

Main Effect: .0875 .96 -.46 -.95 -1.39 .0875 5.73 .18

Center replicates:

$\bar{X} = 7.01 \text{ g}$ SD = $\pm .56 \text{ g}$

(survivors only)

TABLE 3

RANKING OF MAIN EFFECTS AND INTERACTIONS
OF EXPERIMENT #1

<u>VARIABLE</u>	<u>EFFECT</u>	<u>CENTER REPLICATE</u>
7 (FOOD)	5.73	
47	2.84	
5 (♂ PRESENCE) (HELPS)	-1.39	$\bar{X} = 7.01 \text{ g}$
25	1.37	
45	1.33	S.D. = .56
247	1.18	
2 (WEIGH FREQ.)	.96	
4 (REMOVAL OF YOUNG)	-.95	

TABLE 4

RESULTS FROM 2⁸⁻⁴

SIGNIFICANT REMAINING VARIABLES FROM EXPERIMENT 1

2

4

5

7

WEANED
TOTAL
YOUNG
BIOMASS
(g)

RUN #	NEW #	(WEIGH FREQ.)	(REMOVAL OF YOUNG)	(MALE PRESENCE)	(FOOD)	AVE. WT. (g)	START WT. (g)	WEANED TOTAL YOUNG BIOMASS (g)
16	(1)	—	—	—	—	4.87	18.6	14.6
7	(2)	+	—	—	—	5.18	17.7	20.7
11	(3)	—	+	—	—	0.	25.3	0.
4	(4)	+	+	—	—	0.	18.2	0.
6	(5)	—	—	+	—	0.	16.4	0.
13	(6)	+	—	+	—	5.10	15.9	10.2
1	(7)	—	+	+	—	0.	17.6	0.
10	(8)	+	+	+	—	0.	17.7	0.
2	(9)	—	—	—	+	9.0	21.7	45.0
9	(10)	+	—	—	+	7.3	16.7	21.9
5	(11)	—	+	—	+	8.75	25.4	17.5
14	(12)	+	+	—	+	8.5	22.0	17.0
12	(13)	—	—	+	+	4.78	21.4	19.1
3	(14)	+	—	+	+	5.62	19.2	22.5
15	(15)	—	+	+	+	6.8	16.4	13.6

Table 1 defines the variables and levels used in the first experiment. Table two shows the experimental design and the results of that experiment in the right hand column, where the average weight of the young at 21 days (typical weaning age) is recorded. The main effect of each variable is noted below the variable columns. It is determined by dividing each column sum (0.7 in the case of variable 1) by the number of pairs of experiments (8). The main effect is a measure of how far from the mean the variable in question "pushed" the young. The standard deviation (0.56) is actually two standard deviations, so any main effect greater than 0.56 likely is significant, if large factor interactions are small. Table 3 ranks remaining main effects and interactions, since variables 1,3,6 and 8 all apparently have no effect at the levels used. The four variables that dropped out reduce the design to a full factorial in the other four variables, allowing one to compute the higher order interactions. Table 4 shows the results with the experimental design rearranged in standard form. It is apparent that by examining the amount of food column (variable 7) and the average weight of young that there is a substantial effect of limited food (- coding) on survival of young.

The lack of effect of water in causing reduction in reproductive capacity caused us to repeat the design, but with lowered levels of water. The design and results of the second experiment are presented in Table 5. This time water was clearly the most influential variable in survival of the young. The presence of a nestbox was the next most important variable. Whether the nest box eases the "stress" on a female already stressed or provides a

Table 5

Experiment #2

P. maniculatus

Variable definitions and levels

Variable	(-) Level	(+)	Center Replicate
1. Sprouts	none	ad lib	1/2 ad lib
2. Weigh frequency	1/day	1/3 days	1/2 days
3. Nestbox	none	present	no roof
4. Remove young	no	leave 3	leave 2
5. Photoperiod	8L:16D	16L:8D	12L:12D
6. Exercise wheel	locked	unlocked	locked every other
7. Amount of food	80% ad lib	ad lib	90% ad lib
8. Amount of water	40% ad lib	80% ad lib	60% ad lib

Table 5 (cont.)
 2^{8-4} design; trying 2 replicates/run;
 2 center replicates/block

Results										21 day young Average	Total # Young
Block #	Run #	1	2	3	4	5	6	7	8	wt. (g)	
1	2	+	-	-	-	-	+	+	+	5.57	9
	10	-	+	+	+	+	-	-	-	4.45	2
2	5	-	-	+	+	-	-	+	+	4.30	1
	13	+	+	-	-	+	+	-	-	1.13	2
3	6	+	-	+	-	+	+	-	+	4.41	7
	14	-	+	-	+	-	-	+	-	1.55	2
4	1	-	-	-	+	+	-	-	+	1.95	1
	9	+	+	+	-	-	+	+	-	3.41	7
5	7	-	+	+	-	-	-	-	+	3.43	2
	15	+	-	-	+	+	+	+	-	1.70	2
6	4	+	+	-	+	-	+	-	+	5.75	3
	12	-	-	+	-	+	-	+	-	3.29	5
7	3	-	+	-	-	+	-	+	+	3.24	2
	11	+	-	+	+	-	+	-	-	2.35	5
8	8	+	+	+	+	+	+	+	+	7.23	4
	16	-	-	-	-	-	-	-	-	1.65	1

$\frac{7.69}{8}$ $\frac{4.97}{8}$ $\frac{10.33}{8}$ $\frac{3.15}{8}$ $\frac{-.61}{8}$ $\frac{7.69}{8}$ $\frac{5.17}{8}$ $\frac{16.35}{8}$

Main Effect: .96 .62 1.29 .39 -.08 .96 .65 2.04

Center replicate:

$\bar{X} = 4.54g$ S.D. = $\pm 1.16g$

more humid microenvironment and thereby reduces water loss remains to be determined. At this point I discovered that the experimental design from the textbook was partly wrong for variable 6. The effect of the error was to confound variable 6 with variable 1. We are rerunning part of the design for experiment 1 and 2 to remove the confounding pattern. Needless to say, we do all our own experimental design now.

Results of the center replicate experiments are very revealing. The only variable of major significance to change was the level of water given. Figure 1 is a plot of the mean and standard deviation of the percent survivorship for litters. As one can see, the response appears sigmoidal. It is also apparent that there is sufficient variation among females in their reserves or physiology or both so that some are able to carry all their young through weaning, even though there is severe water stress.

Figure two shows how the weight of the young varies as a function of water stress on the female. Even though the sample sizes are not very large yet, the trend is for a smaller average size at 21 days (normal weaning) down to survival sizes that are the same as some that died in other experiments. The variance in size of the young also increases with increasing water stress, reflecting possibly not only differences in females, but possibly also the young in either obtaining food in short supply or processing it more efficiently. Whatever the mechanism, these preliminary data suggests that wild populations of deer mice are capable of withstanding reduction in available water on the order of 50% before coming close to reproductive failure. There are obvious implications here for

Experiments 1+2

FIGURE 1

Center replicate data

P. maniculatus

22°C

90% ad lib food

Experiments 1 + 2
 Center replicate data
P. maniculatus

FIGURE 2

22°C

90% ad lib food

genetic selection with prolonged water deprivation. It appears that even with 90% ad lib food, if environment has little or no free water, these mice cannot reproduce.

Appendix 1

Note: The material quoted here is from Box, G.E.P., J.S. Hunter and W.G. Hunter, Statistics for Experimenters, class notes, Stat. 424. 1976

1. Advantages of factorial vs "one-factor-at-a-time" experimental arrangements.

"The method in which factors are varied one at a time, with the remaining factors held constant, is sometimes regarded as the only correct way to conduct research. The method provides an estimate of the effect of a single variable at selected fixed conditions of the other variables. However, for such an estimate to have more general relevance; it is necessary to assume that the effects would have been the same at other settings of other variables. The assumption is thus that at least over the ranges of current interest the variables act on the response independently." Box, Hunter and Hunter (1976) demonstrate that "(1) if the variables do act independently the factorial does the job with much less effort and (2) if the variables do not act independently the factorial, unlike the one-factor-at-a-time design, can detect and estimate the dependence.

Gain in precision

There is a gain in precision if variables act independently when a factorial (fractional or full) is used rather than "one at a time" methods. For example, to achieve the same precision in the estimate of, say, a temperature effect on product production with levels of concentration and catalyst as the other variables, the one-factor-at-a-time experiment would require eight runs, four at each level of temperature with all the observations made at some arbitrarily fixed levels of concentration and catalyst. Thus to obtain estimates of

the effects of these variables with the same precision as is provided by the 2^3 factorial design, the one factor at a time method would require 24 runs - a threefold increase. In general for k factors a k -fold increase would be required. Some economy can be introduced by using the same base from which to make all changes in the one-factor-at-a-time design. Even with this arrangement, however, the one-factor-at-a-time design requires $(k+1)/2$ times as many runs as the factorial."

2. Sequential vs comprehensive approach using factorials.

"It may appear reasonable at first sight to adopt a comprehensive approach in which the entire range of every factor is investigated. The resulting design might perhaps consist of all combinations of several levels of all factors. However, this is usually an inefficient way to organize experimental programs. This fact is related to the paradox that the best time to design an experiment is after it is finished, the converse of which is that the worst time is at the beginning when least is known.

To use a comprehensive approach in which the entire experiment is designed at the outset, it is implicitly assumed that the following are known:

1. which variables are the most important and hence should be studied
2. over what ranges the variables should be studied
3. in what metrics the variables and the responses should be considered (linear, logarithmic, reciprocal)
4. what transformations are most meaningful.

In most real investigations, the experimenters will know least about these concerns at the beginning and will gradually learn about them

as the program evolves.

Scientific investigation is a learning process which is usually most rapidly advanced by a sequential strategy. This points to the desirability of the performance of a sequence of relatively small designs. As results from each group of experiments become available questions will be raised, which may or may not have been in the mind of the investigators when the experiments were first planned. Thus as an investigation proceeds the general location of the experiments in the space of the variables may change. Typically, it will become apparent that some of the variables included in the study, because they were thought to be important, are actually not important and that other variables, thought unimportant at first, are quite influential. Thus the subspace of the factor space being explored can change."

"In recent years theories of so called "optimal design" have increasingly appeared. Unfortunately these tend to vastly oversimplify the experimental situation. In such studies it is usually implicit that

1. the variables of interest, the region of experimentation, and the metrics and transformations in which the variables were to be considered are all known and the only problem is to find at what points in the factor space experiments should be run. This oversimplification is further oversimplified by supposing that
2. except for experimental error, the mathematical model that describes the physical phenomenon is also known exactly. Furthermore, it is unrealistically assumed that

3. the experimenter's purposes, which are usually multifaceted, can be summarized in terms of a single quantity called the design criterion. Accordingly some experimental design criterion is "optimized", a comprehensive design is produced, and the problem is neatly solved.

In fact all these characteristics of the experimental environment assumed known by the theorist are usually unknown. Experimenters are concerned just as much with finding out which variables should be studied, over what ranges, in what metrics and transformations, and what model might be appropriate as they are with the narrower aspects of the experimental design problem of current concern to theoreticians.

It is extremely difficult, if not impossible, to define the full dimensions of an experimental problem ahead of time. But since human beings are familiar with the learning process and have survived as far as they have because of the efficiency of this process, the level of judgment necessary for reasonable success with a sequential approach is present in most human beings. The approach we adopted, therefore, is that experimentation is part of the learning process, that the learning process is the sequential accumulation of knowledge and hence that our experimental designs and strategies must be adapted to this natural sequential process."

The sequential experimental process can be represented in three dimensions in Fig. 1. The first series of experiments (1) establishes contours leading to a maximum. The next series of experiments (2) is deliberately placed slightly beyond the intended maximum to confirm a high point. The third series of experiments is placed in the space

where the peak is expected. (3) Outliers are added for maximum resolution of the surface near the peak to obtain a central composite design which is extremely efficient and reliable.

Center replicates in the design serve two functions: 1) they provide a measure of the variance of the experimental results without having to have replicates on the corners (though there may be replicates, if so desired for increased accuracy). 2) they reveal whether any two variable relationship is non-linear. If there is a linear relationship, in the cube, the center replicate values should be intermediate between any two corners of the cube. If the center replicate values are very high or very low, i.e. close to one corner, but not the other, or even outside the cube, a curvilinear relation exists and an iteration would be done with more levels for finer resolution. Since any curve can be approximated by straight line segments, the fractional designs apply to both linear and non-linear relationships. It is only necessary to insert enough levels on a given experiment to satisfactorily approximate a curved relationship. In summary, one assumes initially linear relationships between levels and between variables if there is no prior knowledge. If there are non-linear relationships, the center replicates will reveal them. More interactions with more levels may then be added only as necessary.

Advantages of fractional over full factorial designs

There tends to be a great deal of redundancy in a full factorial two level design with k variables (2^k design) if k is large. "The redundancy is in terms of an excess number of interactions that are estimated and sometimes in an excess number of variables that are studied. A certain amount of redundancy is desirable but excessive

redundancy is wasteful. With fractional factorial designs excessive redundancy can be eliminated. For example, instead of running a 2^5 design, a 2^{5-1} design can be constructed leading to 16 experiments instead of 32." (A simple 2^3 vs 2^{3-1} design is illustrated in Table A-1.) If one of the variables turns out to be inert, it can be shown that the 2^{5-1} design will generate a complete factorial in the four effective variables no matter which variables they turn out to be. If all five variables turn out to be important, the only information that can not be resolved is the highest order interaction, i.e. the interaction of factors 12345. If it is desired to obtain that information, one need only reverse the signs of the design for the confounded variable, do the 2^{5-1} experimental design again to get full resolution of all interactions with a given variable for any version of fractional design. There are other design options for modifying the sign pattern of the design that will yield additional resolution for many variables rather than just one when using smaller fract:

Fractional factorial designs offer great flexibility and economy of effort when large numbers of variables must be explored. The caution that must always be present is that higher order interactions are always confounded with main effects in fractional designs. Higher order interactions may be of opposite sign and may make it appear as though the main effects are insignificant, since the effects are summed in confounding. In practice higher order effects are usually much smaller than main effects and of the same sign. "Saddles" are a possible geometry that could confuse the analysis, which is why center replicates are very important since they provide a third intermediate point on a line between any opposite points, thereby signalling the presence of a curvilinear function.

Table A-2 is a resolution table showing the level of resolution obtainable for up to 11 variables. The number of variables in an experiment are across the top of the table and the number of experimental runs in an experiment are listed down the left side of the table. The Roman numerals indicate the level of resolution. For example, III indicates main effects confounded with 2 factor interaction IV indicates main effects confounded with three factor interactions and higher, but free of two factor interactions (usually the minimum desirable resolution). V indicates main effects confounded with four factor interactions and higher, but main effects free of confounding with two and three factor interactions. The subscripts on the capital F's indicate the number of replications of a full factorial design with that number of variables and runs. As can be seen, when a variable can be shown to be not significant in a fractional design, the design usually reverts to a higher resolution design without any further experimentation.

In summary, higher order interaction resolution is traded for fewer experimental runs and resolution of main effects or main effects and lower order interactions only. Further resolution for any given variable is obtained by another run of the same design, but with signs in the variable column of interest reversed. When experimentation is sequential the fractions may be used as building blocks. We can always build up the full factorial design if this is necessary, but frequently it is not. Since there tends to be a hierarchy in the size of effects of higher and higher order interactions, fractional designs are more efficient than full factorials for large numbers of variables. On the whole, in absolute magnitude, main effects of

variables tend to be larger than two-factor interactions, which are larger than three-factor interactions and so on. Thus interactive designs that allow one to explore main effects first and then add more resolution as the need arises are far more efficient than looking at all possible interactions in the first experiment.

Table A-1

A 2^3 design vs a 2^{3-1} design

Variable →	1	2	3	Variable →	1	2	12 + 3
Run #				Run #			
1	-	-	-	1	-	-	+
2	+	-	-	2	+	-	-
3	-	+	-	3	-	+	-
4	+	+	-	4	+	+	+
5	-	-	+				
6	+	-	+				
7	-	+	+				
8	+	+	+				

The -, + notations are codes for the 2 experimental levels of each variable.

Sample Variables	(-) level	(+)	Center Replicate
1. Amount of food	80% ad lib	ad lib	90% ad lib
2. Amount of water	80% ad lib	ad lib	90% ad lib
3. Amount of sprouts	none	ad lib	1/2 ad lib

Measured variable (experimental result): g biomass weaned by a female in each run

Figure 1

A simple three variable series of experiments in time starting with (1) then (2) and last (3) to determine a response surface as a function of three variables. The numbered dots in (1) indicate experimental values obtained for the specified variables and their levels, e.g. in (1) 30 units are produced for +, +, + levels of all 3 variables.

Note: The levels in (2) and (3) are different magnitudes as represented by the positions of the boxes in three dimensional space.

Experiment 3

Experiment 2

CENTER REPLICATES

PATH OF
STEEPEST
ASCENT

Experiment 1

Figure 1

Part III "Cactus" experiments.

Last spring our data for indoor and outdoor experiments were such that we could draw the following contours for enhancement of convection as a function of increasing spine density (Fig. 1). Last fall, after building additional "cacti" for better resolution, we obtained the results in Fig. 2. The most curious result is the substantial enhanced convection at critical spine densities with very little "extra" enhancement at densities close to these "super" cacti. To illustrate the magnitude of change, Figure 3 shows the enhancement at 1 m/s and 2 m/s for all "cacti". The mechanism for this added enhancement is uncertain at present. We are in the process of trying to resolve this mystery.

Figure 2

FIGURE 2

Literature cited

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