## Colin Pittendrigh in his final years at Princeton University

The manuscript associated with these remarks is an abbreviated version of my Ph.D. dissertation, earned in 1969 under the guidance of Colin Pittendrigh (Pitt) at Princeton University. The manuscript was not published, or more accurately was rejected, by Science magazine in 1976. I chose the subject because I was interested in the ecology of flies of the genus Drosophila, and I wanted to generate evidence that natural environmental conditions are a source of selection for the well known circadian eclosion rhythm of these animals. All species of Drosophila tested have a circadian eclosion rhythm that causes adult flies to emerge from the pupal case only in the first few hours after dawn. I selected one species of Drosophila native to the western U.S., and a second species native to the eastern U.S, and exposed both to natural and extreme saturation deficit regimes. Saturation deficit is a measure of the evaporative power of the atmosphere at a particular temperature and relative humidity. I wanted to find out if Drosophila in nature more successfully eclose in the early morning because that is the daylight time when the saturation deficit is usually at a minimum. I found this to be true for both species when exposed to their natural environment. In the laboratory, I found a positive relationship between eclosion success and low saturation deficits. Pitt steadily encouraged me in this work, reviewed the manuscript and made helpful suggestions before I submitted it for publication.

I arrived at Princeton in the fall of 1965, eager to meet my new scientific mentor, Colin Pittendrigh. I had developed an interest in circadian rhythms as an undergraduate, and decided he was the person I wished to work with. I closely watched and learned from him, the most important person in my professional development.

As it happened, Pitt had just become Dean of the Graduate School, a major administrative job with offices in historic Nassau Hall, far removed from his lab physically and psychologically. His lab was very busy, with several graduate students, post docs, and fulltime laboratory assistants and technicians. The suite of rooms in 19th Century Guyot Hall was a beehive of scientific activity, or perhaps I should say a fly swarm. Much of the research on circadian rhythms revolved around one organism, Drosophila pseudoobscura, a western North American species of fruit fly. Among the students in the lab at that time were the late Arthur Winfree of the University of Arizona, a mathematical biologist and MacArthur Fellow who wrote The Geometry of Biological Time, and Jerry Feldman of Swarthmore College, who has published many papers on molecular circadian clocks. There were often visitors to our laboratory. Some were colleagues from other universities. On occasion, there would be reporters and film crews interviewing Pitt on subjects as diverse as the possibility of extraterrestrial life, and federal support of scientific research. We were once visited by Jürgen Aschoff, who together with Pitt and Erwin Bünning laid the groundwork for modern studies of chronobiology. Aschoff met individually with every researcher in the lab, and listened with interest as we each explained our work.

My first research idea was a hypothesis that the blind cave dwelling crustacean, *Orchonectes pellucidus*, long isolated underground in Mammoth Cave, Kentucky, would have retained circadian rhythms. I planned to bring animals into constant laboratory conditions, and measure their cyclical physical and physiological functions. I spent my first year planning that project, only to discover that graduate student Thomas Jegla had just completed those very experiments for his dissertation at Yale. He published his results, showing that the crayfish do express free running circadian rhythms in constant conditions, in the Journal of Experimental Zoology in 1968. I had to start over with another idea.

Pitt as always was supportive, and we began to discuss research alternatives. I formulated a question that would use *D. pseudoobscura*. I wanted to make up for lost time, and this choice meant that I could take advantage of equipment and procedures already in use in his laboratory. I was off and running. My experimental design required exposing fly pupae to natural environmental conditions. I did this near Princeton using a small laboratory built for me inside a century old farmhouse barn on property owned by the University. This field lab must have been a costly construction project. I do not know because after I asked about it, in short order it was built for me as per my specifications. It was Pitt, of course, who arranged and paid for that. He never told me how.

The following year I needed to take my experimental flies to a western U.S. climate, where they naturally occur, to find out how precisely the well studied circadian eclosion rhythm exhibited in our Princeton laboratory is entrained by natural temperature and light cycles. Pitt sent me to the Rockefeller University in New York City to confer with Theodosius Dobzhansky, at the time the grand old man of evolutionary genetics. Pitt pointed out to me that Dobzhansky was my intellectual grandfather, since he had been Pitt's major professor at Columbia. Pitt referred to him as "the old boy." Dobzhansky had been working for many decades with *Drosophila*, and had done so in the American West. The first thing he said to me was "So here is the young man who does not sign his letters." I had typed my letters to him, and in my callow years I did not realize it was good form to include a signature. He made this remark with a kindly smile on his face, because Dr. Dobzhansky was a markedly courteous, indeed courtly, person who greeted me as a colleague. He listened with interest as I explained my research, and sought his advice. In his pronounced Russian accent, he addressed me as "Meester Quinn." He showed me around his lab, where a number of researchers, who were familiar to me only from their publications, explained their research programs. Dobzhansky then invited me to present a seminar on my research to his assembled laboratory colleagues and staff. I asked him when, expecting a later date, and he replied "this afternoon at four," two hours later. How could I say no, even though I was completely unprepared and quite nervous about an audience who had been famously studying Drosophila for decades? I somehow gathered my wits and made a coherent presentation, with arm waving in place of slides. Afterwards Dr. Dobzhansky invited me to dinner with him and his postdocs at the Rockefeller University, a place where men were required to dress for dinner in a coat and tie. I came that day dressed as a humble graduate student. The good doctor arranged for one of his post-docs, a tall man, to loan me the proper garb, so I ate and conversed with the sleeves of my coat jacket rolled up. Very elegant. Dr. Dobzhansky recommended that I do my field work at the Southwestern Research Station, a facility in Arizona operated then and now by the American Museum of Natural History. He had worked there, where he was remembered for travelling around to his field sites on horseback. The Biology Department paid for my travel expenses, but not for a horse, and Pitt had the experimental machinery I needed built and shipped to Arizona. Colin Pittendrigh had one of the most remarkable scientific minds that I have ever encountered. He grasped complex ideas immediately, and often foresaw their implications for research just as quickly. I always carefully prepared for my meetings with him in his distant administrative offices, because I knew that he could process and analyze whatever I said as quickly as I could explain myself. After our meetings, I would sit down and write out everything he had said, while it was fresh in my mind.

His glittering verbal and analytical abilities were equally matched. When he spoke formally he was elegant, and he knew how to tailor his remarks to his audience. He enjoyed explaining evolution in the general sense. I saw him do that at a public lecture in the Princeton Public Library. I was taken by his lecture because he stated his ideas so clearly for a very general audience. On another occasion, I saw him explain evolutionary principles, in one hour, to a very large undergraduate class. His remarks were so engaging that when he concluded the entire class stood and gave him a standing ovation. That was the only time I saw that happen in any Princeton undergraduate class.

As I was pushing toward the completion of my dissertation in the spring of 1969, I learned that Pitt was leaving Princeton, as was I. He moved to Stanford University where he continued his research, and assumed a new set of important administrative duties. He of course continued his scientific research. He once told me that he was concerned that he was living on his "scientific capital," implying that his administrative responsibilities were causing him to neglect scientific research. I did not see that happen during his final Princeton years.

I feel extremely fortunate to have studied under Pitt. He was supportive, and by his example he helped me learn to think and work as a scientist.

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# SELECTIVE PRESSURE RESPONSIBLE FOR THE CIRCADIAN ECLOSION PERIODICITY OF <u>DROSOPHILA</u>

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### ABSTRACT

Five species of <u>Drosophila</u> eclose successfully more often when exposed to low, rather than high, saturation deficits. Flies are affected by water vapor immediately before, rather than during or immediately after, eclosion. Saturation deficits constitute a strong source of natural selection for confining eclosion to the dampest time of day.

#### TEXT

Adult <u>Drosophila</u> emerge from the puparium only during the morning hours. When populations of <u>Drosophila</u> pupae are exposed to either a natural light cycle or a laboratory photoperiod, almost all adults emerge during the six hours following the onset of light. Despite extensive study of the circadian eclosion rhythm of <u>Drosophila</u>, relatively little attention has been given its adaptive significance. The atmosphere is coolest and wettest in the morning, and Pittendrigh suggested that the emergence behavior permits the young fly to minimize evaporative water losses (1). To test the physiological importance of atmospheric water vapor, pupae of several <u>Drosophila</u> species were exposed to high and low saturation deficits just before emergence.

Populations of <u>Drosophila</u> which pupated within a 24 hour period were collected from culture and allowed to complete development in an incubation chamber which regulated saturation deficit (2). Experiments were conducted at  $20^{\circ}C$  ( $\pm 0.5^{\circ}C$ ) with a 12 hour daily photoperiod. Since all pupae were of similar developmental ages, most eclosions occurred on the same day. Late in the photoperiod preceding eclosion male and female pupae were separated. At the beginning of the following photoperiod, just before eclosions began, half the pupae of each sex were placed at a high, and half at a low, saturation deficit. Six hours later, after most eclosions had occurred, all insects were returned to the incubation

saturation deficit. Before the end of the photoperiod, each test group was scored in two categories; adults capable of flying, and all others. The latter group included adults unable to fly, individuals which became stuck before completely leaving the puparium, and pupae showing no evidence of eclosion. Non-flying adults were fed for 24 hours and retested for flying ability; those which could fly were added to the tally of flying adults. Most adults which could not fly had one or both wings incompletely expanded. Intact pupae were checked again for eclosion after another 24 hours in the incubation chamber. Any which had eclosed were eliminated from the experiment by subtracting them from the total score of unsuccessful individuals. In this way remaining pupae, which were all dead, were distinguished from those few which had not completed development on the test day. Tests were conducted in two kinds of high and low saturation deficit pairs. One pair was the maximum and minimum obtainable for the experimental temperature; the other resembled saturation deficits found during the insect season in the area where the test strain was originally collected (3). The saturation deficit in the incubation chamber, where the pupae developed, was halfway between the maximum and minimum experimental saturation deficits.

Saturation deficit clearly influences the eclosion of <u>Drosophila</u>. With one exception, both sexes of all species studied produced significantly higher percentages of successful adults in moist air than in dry (Table 1). The effect

of a high saturation deficit is especially clear when comparing D. miranda and D. pseudoobscura (Arizona, Series II) results at environmental and laboratory extremes. The two sets of experiments were carried out simultaneously on subsamples of the same populations. In both species a greater difference between high and low saturation deficits produced a larger mean difference in the percentage of successful eclosions. The Arizona race of D. pseudoobscura was tested In the second series of experiments there was a twice. higher percentage of eclosion success at both saturation deficits, and the mean difference between the two percentages was smaller. The cause of this change is unknown. The two series were done a year apart; during the interim the laboratory culture was sometimes as small as a few hundred individuals. Perhaps there were changes in the frequencies of genes responsible for moisture sensitivity. It is known that gene arrangement affects pupal survival in Drosophila (4). These experiments demonstrate the physiological significance of restricting eclosion to the morning hours, when the air is dampest. They also identify the developmental time when moisture is critical. The sensitive time is immediately before, rather than during or immediately after, eclosion because Most pupae which did not produce fully developed adults never opened the operculum (Table 2). Exposure to contrasting saturation deficits produced no statistically significant\_differences in either incomplete eclosions or wing deficiencies.

Exposure to contrasting saturation deficits during the last six hours of the dark period, just before eclosions begin, did not produce consistent differences in eclosion success.

Restricting eclosion to some specific part of the day is a general phenomenon of holometabolous insects. Of 242 species from ten orders, Remmert lists 228 with emergence periodicity (5). The adaptive significance of such timing has been obscure. Pittendrigh thought post-dawn emergence enabled adults to avoid undue desiccation (1). The rate of water loss by young flies is twice that of older individuals (6). There is, however, no evidence that this temporarily high water loss significantly impairs viability of adults exposed to low mid-day humidities. Moreover, Remmert and others have pointed out that minimum saturation deficits occur slightly before, rather than after, dawn (6, 7). Clayton and Paietta have shown that the circadian eclosion rhythm of Drosophila strains which have been in culture for many years are subject to greater artificial selectability than a recently collected strain (7). This implies that morning emergence is maintained by natural selection. Although the total adaptive utility of post-dawn eclosion remains to be seen, these experiments for the first time identify one strong source of natural selection for confining emergence to the time of day when saturation deficits are at a minimum.

In the species of <u>Drosophila</u> studied, high saturation deficits act before eclosion, either on the pharate adult or the pupal case. Evaporative water losses may be harmful to an insect about to undergo the physical exertion of breaking out of the pupal case. It is conceivable that water economy at that time is a matter of gains rather than losses. A pupa may need to absorb atmospheric water vapor shortly before eclosion. Insects which have this absorptive ability require a moist atmosphere to do so (8). It may also be that high levels of water vapor influence the mechanical properties of the opercular seam, allowing the emerging adult to tear it open more easily.

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#### REFERENCES AND NOTES

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- 2. In the incubation chamber pupae were spread so that they did not touch one another on the taut surface of nylon netting. Netting was 2cm above an aqueous solution of sulfuric acid. The chamber was closed by a loose gravity seal and placed inside a larger chamber, similiarly sealed and regulated by sulfuric acid solution. This arrangement allowed slow gas exchange, while preventing the relative humidity of the room from significantly affecting the saturation deficit of the smaller chamber. Measurements with a recording hygrosensor (No. H-170, Hygrodynamics; Inc., Silver Springs, Maryland) showed that the saturation deficit of the inside chamber stabilized within 15-30 minutes of sealing, remaining constant at the level expected from the concentration of the sulfuric acid solution.
- 3. Environmental saturation deficits were calculated from weather records of temperature and relative humidity, using hourly values for ten or more days scattered through the season. The mean saturation deficit for hours 12-17:00

and 1-6:00 were used for maximum and minimum values respectively. Calculations for each of the collecting areas showed these to be the driest and wettest hours of the day.

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- 9. Supported by NASA Contract NASR-223 to C.S. Pittendrigh, N.I.H. Traineeship Grant GM-457, and Grant-in-aid, Biology Department, Princeton University. I than C.S. Pittendrigh for support and critical reading of the manuscript, and acknowledge the support of the late R.H. MacArthur.

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Table 1. Eclosion	succes	ss of Dros	sophila at contr	rasting satura	tion deficits. The	
first five rows ha	ve satı	uration de	ficits similar	to environmer	ital extremes. The	
saturation deficit	s in th	le last f	our rows were th	he maximum and	l minimum obtainable	
at 20 <sup>0</sup> C. Each row	is der	rived from	n trials, usual	ly done on co	secutive days, of	
serial samples of	pupae 1	from the	same population	•		
					Mean % difference	
			Combined		in eclosion success	
			number of	Saturation	between low and	
	Numbe	с И	flies in	deficits	high saturation	
Species	of ti	rials	all trials <sup>++</sup>	(mm Hg)	deficits	
D. miranda	6	м	860	0.7, 5.4	- 1.0	
	0+	4	1506		+ 5.3 #	
D. affinis	ю	9	812	1.7, 8.4	+10.7 *	
	0+	7	1478		+11.4 #	
D. pseudoobscura	б	Ŀ	1902	0.2, 15.4	+11.0 **	
+(California)	0+	Ŀ	1080		+ 9.6 +	
D. pseudoobscura	б	4	740	0.5, 14.3	+11.2 *	
+(Arizona,	0+	4	646		+14.0 **	
Series I)						

D. pseudoobscura	ю	б		1854	0.5, 14.3	+ 2.0 #
(Arizona,	0+	σ		1696		+ 3.8 #
Series II)				•		
D. miranda	٣	~		27/1	ער ער ער	0
	C	J		+	C · / + · C · O	0.7 +
	0+	M		920		+ 8.4 #
D. pseudoobscura	ъ	р		814	0.5.17.5	+ 7.3
(Arizona,	0+	М		762		+ 9.5 #
Series II)						<b>A</b> -
D. algonquin	Ю	Ŋ		296	0.5, 17.5	+13.4 **
	0+	Ŋ		450		+ 8.7 **
D. persimilis	б	ω		1962	0.5, 17.5	+ 5.0 **
	0+	TO		2346		+ 3.5 **
++The number of pul	pae in	eac	h trial,	within a a	series, was appro	oximately equal. <sup>+</sup> The
races of <u>Drosophil</u>	a pseu	Idoob	scura we	re origina]	lly collected at	Mather, California
and Portal, Arizons	д. *	-01	using a	I one-sided	Student's t tes	t, each trial consti-
tuting a pair. I a	assume	d th	e low sa	turation de	eficit would prod	duce a greater per-
centage of flying a	adults	tha	n the hi	gh. **P<.(	03. <sup>#</sup> P<.08.	

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> The fate of pupae subjected to saturation deficit tests. Table II.

			PERCENTAGE F	3RE AKDOWN	
	•				
		Flying	Wing	Incomplete	No
Species	Total #	adults	deficiency	eclosion	eclosion
Drosophila pseudoobscura	8670	84.06	1.57	3.73	10.65
Drosophila persimilis	3015	87.66	3.65	1.92	6.77
Drosophila miranda	4889	80.81	3.56	4.46	71.11
Drosophila algonquin	632	64.87	2.53	4.43	28.16
Drosophila affinis	2288	27.84	1.70	15.82	54.63
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