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Selection for Circadian Eclosion Time in *Drosophila melanogaster*

Abstract. *Early and late eclosion strains were developed from* Drosophila melanogaster *cultures. The Oregon-R parent strains (isolated in 1925) showed significantly more selectability than the W² parent strain collected at the beginning of this study (1971). This is consistent with the hypothesis that the selective advantage of circadian behaviors is reduced in laboratory conditions.*

Many characteristics of circadian rhythms have been investigated in detail, for example, phase response (*1*), free-running period (*2*), temperature independence (*3*), and numerous physiological factors (*4*). Maintenance of these characteristics in various taxa [such as plants, protozoa, insects, and mammals (*1, 5*)] implies that they have general adaptive value.

Bunning (*6*) crossed plants with different period lengths and suggested Mendelian segregation of period length, because intermediate periods occurred in F_1 plants and the original periods appeared in following generations. The same was evident in Danilevskii's work with the butterfly *Aeronycta rumicus* (*7*).

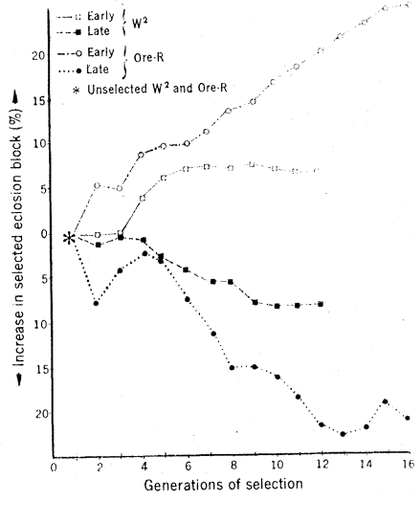

Fig. 1. Selection for early and late eclosion strains of *Drosophila melanogaster* wild-caught (W^2) and laboratory-reared (Ore-R) populations (*13*). The values on the ordinate are the percentages eclosing in block 1 of the early strain and block 3 of the late strain minus the percentages eclosing in these collection blocks of the unselected stocks (Table 1). The mean number per data point is 362 flies; the range is 153 to 652.

Konopka and Benzer (*8*) showed that free-running periods in *Drosophila* exhibit classical Mendelian characteristics and have further localized the mutant gene or genes affecting period length to a very short segment of the X chromosome. These mutant stocks were developed by using the mutagen ethyl methanesulfonate. It is likely that flies with varying period lengths also occur in nature. Maintenance of 24-hour periods in natural populations implies selection against other periods. In this report we discuss the selectability of wild and laboratory-reared populations of *Drosophila* for early and late eclosion.

Selection lines for early and late eclosions from the pupae case were developed by using laboratory and wild populations of *Drosophila melanogaster*. The laboratory population was of the Oregon-R strain (Ore-R) isolated in 1925 by D. E. Lancefield (*9*) and obtained from A. Yanders (Michigan State University) in 1966. The wild population (W^2) was collected at the beginning of this study (September 1971) in Walla Walla, Washington.

The selection schedule consisted of three consecutive 4-hour collection blocks (periods) beginning 6 hours before and ending 6 hours after dawn. Flies eclosing in blocks 1 and 3 were used as breeding stock for successive generations of the early and late lines, respectively. Block 2 flies were counted and discarded. All flies were reared in 31-g shell vials containing 10 cm^3 of media and maintained at 25°C in a cycle of 12 hours of light followed by 12 hours of darkness (LD 12 : 12).

If selection pressures have been altered by laboratory rearing, one might expect differences in (i) the initial eclosion band shape and (ii) the responses to artificial selection when laboratory-reared flies were compared to wild-caught flies. No difference was seen in the initial eclosion band (Table 1, $P > .975$ by χ^2 analysis). The collection blocks were broad (4 hours), and shorter blocks (1 hour) might have shown a subtle difference. On the other hand, selection for early and late eclosion did demonstrate striking differences in variability of the two parent strains ($P < .01$ by sign test). This is true for comparisons of both early and late eclosion. In both cases the laboratory strain showed greater variability, a result suggesting a relaxation of selection pressures (Fig. 1).

These results are, in one respect, counterintuitive. Forty-seven years of laboratory culturing have no doubt subjected the Ore-R strain to considerable inbreeding, which reduces genetic variability (*10*). In addition, selective pressures different from those in which the species has developed tend to truncate gene distribution. One might, then, expect laboratory rearing to reduce genetic variability unless selective pressures in natural conditions are even more limiting than the forces of inbreeding and artificial (laboratory) selection.

Pittendrigh (*11*) suggested that humidity levels in nature are effective in selecting for eclosion times. Eclosion in *D. pseudoobscura* is more limited to early dawn, when humidity is highest,

Table 1. Eclosion of unselected (generation 1) laboratory (Ore-R) and wild (W^2) populations of *Drosophila melanogaster* in three consecutive 4-hour collection blocks. Dawn of the LD 12 : 12 cycle occurred 2 hours into block 2 (*N*, number of flies eclosing).

Collection block	Eclosion in strain			
	Ore-R		W^2	
	N	%	*N*	%
1	73	11.2	43	10.8
2	503	77.3	310	77.5
3	76	11.6	47	11.8

than is eclosion in *D. persimilis* (the less arid species). The theory is that the wings of flies eclosing when humidity is low are likely to dry before straightening. Flies with abnormal wings are not as motile, nor are they as effective once they locate a mate (*12*). Flies cultured in the laboratory do not experience desiccation after dawn (that is, when lights are on) because the stoppered bottles maintain high humidities continuously. The absence of humidity cycles in laboratory cultures can account for only part of the increased variability of the Ore-R strain. In natural conditions humidity is relatively high for some time preceding dawn; therefore, humidity cycles would not account for the difference between early eclosion strains of the laboratory and wild populations.

DALE L. CLAYTON
JOHN V. PAIETTA
Department of Biology,
Walla Walla College,
College Place, Washington 99324

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17 July 1972

S-100 Protein Synthesis by Isolated Polyribosomes from Rat Brain

Abstract. *The radioactive proteins synthesized in a cell-free system and released from polyribosomes from rat brain were analyzed for the presence of a protein found only in the nervous system. Polyribosomes from rat liver were also studied to demonstrate the organ specificity of the radioactive product synthesized in vitro. The S-100 protein constituted 0.15 percent of the radioactive proteins released from brain polyribosomes.*

The S-100 protein, an acidic cytoplasmic protein, is found only in the nervous system (*1*). Although its role in neural function is not understood, it provides an organ-specific marker to study macromolecular synthesis in in vivo and in vitro neural systems. Studies of rat brain with in vivo labeling suggested that S-100 had high rates of synthesis and degradation (*2*). However, a reexamination of the turnover rate of S-100 protein in the whole brain of the rat indicated that the degradation of this protein in vivo occurs at about the same rate as that of total soluble protein (*3*). The degradation rates of S-100 and total protein were also similar in clonal lines of glial cells in culture. The measured rate of S-100 protein synthesis in culture was only a fraction of 1 percent of the total synthesized protein (*4*). Previous studies with microsomal or ribosomal preparations from rabbit brain indicated that 15 percent of the labeled, released protein could be precipitated with antiserum to S-100 protein (*5*). Because of these conflicting reports concerning synthesis and degradation, we reinvestigated S-100 protein synthesis in cell-free preparations with rat brain polyribosomes. Our results indicate that only a small percentage of the protein released from polyribosomes is S-100 protein.

Brains and livers from Sprague-Dawley rats were placed into ice-cold 0.25*M* sucrose–NH_4Cl buffer [0.1*M* NH_4Cl, 0.03*M* tris(hydroxymethyl)aminomethane (tris)-HCl, *p*H 7.4, 0.005*M* $MgCl_2$, and 0.005*M* β-mercaptoethanol (B-ME)]. Both tissues were minced, were rinsed several times, and were homogenized with eight to ten strokes of a Teflon and glass homogenizer. The homogenate was centrifuged for 10 minutes at 27,000*g*, and the resulting solution was centrifuged for 90 minutes at 195,000*g*. The supernatant solution from the second centrifugation was placed on a Sephadex G-25 column equilibrated with NH_4Cl buffer. This high-speed supernatant (HSS) was used as a source of enzymes and transfer RNA. To prepare polyribosomes, we rehomogenized the pellet centrifuged at 27,000*g* and centrifuged it again. The resulting supernatant solution was combined with the polyribosomes in the pellet from the 195,000*g* centrifugation. After suspension, the polyribosome solutions were treated with a mixture of sodium deoxycholate (DOC) and Tween 40; the final concentration of the solutions was 1.0 percent DOC, 1 percent Tween with brain polyribosomes, and 1.3 percent DOC, 1 percent Tween with liver polyribosomes. The polyribosomes treated with detergent were kept at 4°C for 10 minutes, and then centrifuged at 27,000*g* for 10 minutes. The supernatant solutions were then layered on 0.5*M* to 2.0*M* discontinuous sucrose gradients (in NH_4Cl buffer), and were centrifuged at 4°C for 24 hours at 130,000*g*. The polyribosome pellets were then rinsed with 0.25*M* sucrose–NH_4Cl buffer, suspended by mild homogenization in the same buffer, and centrifuged at 1000*g* for 10 minutes. The polyribosomes in the supernatant were stored at −70°C until needed.

The cell-free reaction mixture (1.0 ml) to study protein synthesis contained: 100 μmole of NH_4Cl, 30 μmole of tris-HCl, *p*H 7.4, 5 μmole of $MgCl_2$, 5 μmole of B-ME, 1 μmole of adenosine triphosphate (ATP), 1 μmole of guanosine triphosphate, 10 μmole of creatine phosphate, 4 enzyme units of creatine phosphokinase (Sigma), 20 nmole each of 15 amino acids in a mixture, and 20 μc each (*6*) of [^{3}H]leucine, [^{3}H]lysine, [^{3}H]phenylalanine, and [^{3}H]valine. In addition, each reaction contained 6 absorbancy units (at 260 nm) of polyribosomes, and 1.2 absorbancy units (at 280 nm) of HSS. Reaction mixtures were incubated for 60 minutes at 37°C, then chilled to 4°C, and centrifuged for 2 hours at 280,000*g* to separate the released proteins from the polyribosomes.

The amount of S-100 protein released from polyribosomes was determined by a modification of the method of Herschman (*4*). Suitable samples of released protein were placed on Sephadex G-15 columns [equilibrated in a solution of 0.01*M* B-ME and phosphate-buffered saline (PBS) (*7*)] to remove unincorporated amino acids and NH_4Cl.